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PAGE

THE INSECTS OF MONA ISLAND (WEST INDIES).   1

By J. A. RAMOS

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[1] As of date of issue. The publication of the "Journal" had been temporarily suspended on account of the printing difficulties encountered during the war years.
[2] Leave to pursue advanced studies.
[3] Other leaves

THE INSECTS OF MONA ISLAND (WEST INDIES)

By J. A. RAMOS[1]

INTRODUCTION

A better and more complete knowledge of the insect fauna of Mona Island has long been desirable from the standpoint of the study of the distribution of insects in the West Indies and of a more thorough knowledge of the entomological fauna of the Puerto Rican region.

The information about the insect fauna of Mona Island included in this paper has been obtained from two sources. In the first place, the writer has attempted to gather together all the published records of insects from Mona Island found in several scattered publications and papers dealing mainly with the fauna of Puerto Rico. In addition to this, a great number of species has been added to those already known from the island and much additional information gathered from a rather extensive collection of insects obtained from the island in April 1935 and during the spring and summer of 1944.

GENERAL INFORMATION

LOCATION

Mona Island, politically part of Puerto Rico, is located in latitude 18°05' North and longitude 67°55' West, in the south side of the Mona Passage between Puerto Rico and Hispaniola. It is approximately 45 miles west-southwest of Mayaguez, Puerto Rico and 40 miles east-southeast of Punta Espada, Dominican Republic. On clear days, both Puerto Rico and Hispaniola are visible from the island. Three miles to the north-northwest lies Monito Island, a very small and inaccessible rock of no particular interest.

SIZE

The island is roughly circular in shape, with a slight indentation on the north side and an angular outline. It is approximately 6 miles long from

west to east and 5 miles wide from north to south.   The total area is about 14,000 acres.

TOPOGRAPHY

The island is relatively flat, with an average elevation of 150 to 175 feet above sea level.   The highest elevation is found on the Northwest Cape, which is 272 feet high.   The surface consists of two sharply divided levels: the coastal plain and the limestone plateau.   The island is surrounded by a coral reef, so complete, that landing a boat is always dangerous, very difficult and almost impossible during certain months of the year.

The coastal plain comprises about 6 per cent of the total land area of the island, or nearly 900 acres.   About 90 per cent of it is on the southwestern side (Uvero Beach) where much protection from the wind and surf is afforded.   Narrow beaches are also found along the west and south coasts (Sardinera Beach and Playa de Pájaros, respectively), but the north and northeast coasts are cliffs sheer to sea level.   The entire coastal plain is low, generally not over 10 feet above sea level.   It is several miles long and averages about half a mile wide.

The limestone plateau, nowhere less than 100 feet high, comprises the greatest portion of the area of the island, or more than 13,000 acres.   It is bordered nearly throughout by steep escarpments and is accessible at only a few points from the coastal plain.   Its surface is sharp and jagged.

The limestone rock is very porous, which permits much subterranean drainage.   For this reason, there are no springs or rivers on the plateau despite its relatively large extent.   There is, however, some variation in the level of the plateau caused by erosion following rains of heavy intensity.   An example of this is Bajura de los Cerezos, a sink-hole near the center of the island, where subterranean drainage is exceptionally high, and where the washed-in soil permits a richer and more abundant vegetation.

A striking character of Mona Island is the fact that it is honeycombed with numerous caves and caverns, some of considerable size.   Centuries of water seepage through the soluble limestone have been responsible for their development.   They may be seen in the cliffs at any part of the island and a few open out on the surface of the plateau.   These caves were once an important source of guano which was commercially removed from them many years ago.

CLIMATE

The climate of Mona Island seems to be very similar to that of Guánica, on the south coast of Puerto Rico, judging from the scanty available information.   Daily temperatures are high and the precipitation rather low.   A

rain gauge maintained for the last 22 years at the Lighthouse of East Cape has received an average annual rainfall of 40.66 inches.

SOIL

The parent rock is Ponce limestone (Miocene). It gives rise to a soil very similar to shallow phase Ensenada clay. This reddish, pliable and very shallow soil is very sparse and found only in depressions on the surface of the plateau. Its depth varies from 0 to possibly 2 feet in some of the largest depressions.

On the coastal plain, the soil is deeper and more abundant than on the plateau. It consists chiefly of consolidated beach material containing coral and shell fragments.

VEGETATION

Despite the arid climate and the paucity of the soil, the plateau is almost completely covered with an open forest of shrubs and low trees of a considerable number of species. Several species of cacti also inhabit it and show abundant growth at some places. The snowy cactus, *Neomammillaria nivosa* (Link) Britton & Rose, a species which does not occur in Puerto Rico, is plentiful. *Cephalocereus Royeni* (L.) Britton & Rose, several species of *Opuntia* and *Cactus intortus* Mill. are also common. Tall grasses are found on some places, but generally the herbaceous vegetation is not abundant on this level. The browsing of the several thousand wild goats and pigs has undoubtedly influenced to some extent the character of the vegetation of the plateau.

On the coastal plain, probably due to the deeper and more abundant soil and to the moister condition, several species of trees reach a larger size and form a denser and higher forest. The herbaceous vegetation is also richer than on the upper level. This richer vegetation may be also observed, however, on some of the larger depressions on the plateau, where, for the same reasons, a denser forest occurs. An example of this is Bajura de los Cerezos.

On the western and southwestern coastal plains, from Sardinera Beach to Uvero Beach, the vegetation has been modified by cutting and clearing for agriculture and reforestation purposes. The Insular Forestry Service planted, from 1937 to 1939, 420 acres in this region to Australian pines, *Casuarina equisitifolia* Forst.; Dominican mahogany, *Swietenia Mahagoni* (L) Jacq.; and "avelluelo", *Colubrina colubrina* (Jacq.) Millsp. The Australian pine has been very successful on this sandy soil, but the "avelluelo" has failed, especially near the coastline, possibly because the salinity of the soil. The success of the mahogany, which was planted on shallow rocky sites, seems not to be too good either.

Britton 1915: 36 gives a total of 230 species of flowering plants from the island, of which only 2 are endemic. These are a small tree, *Tabebuia lucida* Britton, rather common on the plateau, and *Chamaesyce monensis* Millsp., a small plant occurring also on that situation.

Some of the most common species of plants on the island (Britton 1915: 37–49) are the following: *Ficus Stahlii* Warb., *Coccolobis uvifera* (L.) Jacq., *Coccolobis laurifolia* Jacq., *Pisonia albida* (Heimerl) Britton, *Sesuvium Portulacastrum* L., *Capparis cynophallophora* L., *Capparis flexuosa* L., *Pithecolobium Unguis-cati* (L.) Mart., *Cracca cinerae* (L.) Morong, *Guaiacum sanctum* L., *Guaiacum officinale* L., *Amyris elemifera* L., *Elaphrium Simaruba* (L.) Rose, *Stigmaphyllon lingulatum* (Poir.) Small, *Metopium toxiferum* (L.) Krug & Urban, *Corchorus hirsutus* L. *Gossypium barbadense* L., *Clusia rosea* Jacq., *Canella Winteriana* (L.) Gaertn., *Carica Papaya* L., *Cephalocereus Royeni* (L.) Britton & Rose, *Opuntia Dillenii* (Ker-Gawl.) Haw., *Eugenia buxifolia* (Sw.) Willd., *Plumiera obtusa* L., *Lantana Camara* L., *Tabebuia lucida* Britton, *Guettarda elliptica* Sw., *Pluchea purpurascens* (Sw.) DC., and *Aklema petiolare* (Sims) Millsp.

<div align="center">FAUNA</div>

Although the land fauna of Mona Island is not very rich in number of species, it is however very interesting, and, for this reason, has received much attention from several students of the Puerto Rican fauna.

Amphibians and Reptiles

Schmidt 1928: 8 reports 9 species of amphibians and reptiles from the island of which 6 species are endemic to it. These are a small frog, *Eleutherodactylus monensis* Meerw.; the rock iguana, *Cyclura stejnegeri* Barb. and Noble, probably the most conspicuous and interesting feature of the fauna of the island; a small iguana, *Ameiva alboguttata* Boul.; and three snakes: *Typhlops monensis* Schmidt, *Epicrates monensis* Zenneck, and *Alsophis variegatus* Schmidt.

Birds

Wetmore 1927: 245–598 records 22 species of birds from the island. Of these, the most interesting form is a ground-dove, *Columbigallina passerina exigua* Riley, which occurs only on Mona and on the island of Inagua, in the southern Bahama Islands, the rest being common species in other West Indian islands. Danforth 1936: 100 adds 4 species to Wetmore's list. These are two North American migrants, the ani, and the Hispaniolan race of the sparrow hawk, *Falco sparverius dominicensis* Gmelin.

Mammals

Two species of bats which are widely distributed throughout the Greater Antilles and the Virgin Islands, *Noctilio vespertinus mastivus* Dahl and *Mormoops blainvillii* Leach, are the only mammals known from Mona Island (Anthony 1926: 208).   They are not common and are found in the numerous caves that occur all over the island.

Wild goats and pigs are abundant on the plateau and together with the large number of scaled and white-headed pigeons present during certain months of the year, constitute an important attraction for sportsmen and hunters from Puerto Rico.

AGRICULTURE

Because of the scant rainfall, poor soil, and great distance from markets, agriculture has never proven practical on Mona Island.   A small area on the southwestern coastal plain was once cultivated and it is said that cotton, papayas, and watermelons were successfully produced.   Pasturing has been more feasible but has been done only to the extent needed by the few to whom the island was leased in the past.

HISTORICAL RÉSUMÉ

The earliest published records of insects from Mona Island appear to be those of the elaterid *Adelocera rubida* Schwarz and of the phasmid *Lampomius bocki* Brunner and Redtenbacher, described from the island in 1902 and 1908 respectively.   Since a German concern was engaged for several years, from the end of the last century to the beginning of the present one, in removing guano from Mona Island, it appears probable that the material from which these two species were described was secured by somebody in the German personnel of that concern, who sent it to museums in his homeland.

Mr. E. G. Smyth and Mr. R. H. Van Zwaluwenburg appear to have been the first entomologists to visit the island and collect insects there, going by sailboat from Mayagüez in December 1913.   The few records of insects collected by Smyth at that time on Mona are listed in the accession catalogue of the Agricultural Experiment Station of the University of Puerto Rico at Río Piedras under the numbers 1300 to 1399 in 1913.

Until 1914 when the explorations for the Scientific Survey of Puerto Rico and Virgin Islands, under the combined auspices of the New York Academy of Sciences, the American Museum of Natural History, and the University of Puerto Rico, were initiated, Mona Island was practically unknown and unexplored entomologically.   During February 21–26 of that year, a small party of scientists visited the island for the purpose of exploring it

and collecting plants and animals. Dr. Frank E. Lutz, of the American Museum of Natural History, was among the members of that party. During the 5 days he spent on the island he collected rather intensively, contributing much to our present knowledge of the insect fauna of Mona. Many of the new species of insects that have been described from the island have been from the material secured by him on that occasion.

In April 1935 the writer accompanied the late Dr. Stuart T. Danforth on a 3-day trip to Mona Island, primarily for the purpose of studying and collecting birds. A small collection of insects made by him on that occasion is reported in this paper for the first time.

Mr. Francisco Sein Jr. was on Mona Island in August 1926, and Dr. George N. Wolcott visited it by airplane on January 24, 1940, to advise regarding an extensive outbreak of thrips on onions being grown on the coastal plain.

Dr. Luis F. Mortorell, now an entomologist of the Agricultural Experiment Station of the University of Puerto Rico, obtained determinations of insects collected on Mona in March 1937, by Mr. M. A. Perez of the Insular Forestry Service. On August 4–7, 1939 and March 29–April 4, 1940, he collected rather intensively there, adding much information about the insects of the island. He is responsible for many of the records from Mona in the accession catalogue of the Agricultural Experiment Station of the University of Puerto Rico for the years 1939 and 1940 as reported by Wolcott 1941: 33-158.

Professor Virgilio Biaggi, Jr., of the Biology Department, College of Agriculture and Mechanic Arts of the University of Puerto Rico, was on an expedition from the Institute of Tropical Agriculture of Mayaguez, Puerto Rico, that visited Mona Island during March 2–7, 1944 for the purpose of collecting plants and animals. Although he was primarily engaged in collecting specimens of birds, reptiles and amphibians, Professor Biaggi was able to make a small collection of insects for the writer during his visit.

On April 1–7, 1944 Dr. George N. Wolcott, Dr. Luis F. Martorell, and Mr. Jorge Serralés, of the Agricultural Experiment Station of the University of Puerto Rico, and the writer visited the island and collected intensively along the western and southwestern coastal plains and on several places on the plateau.

During the summer of the same year, several persons visited Mona and kindly collected insects for the writer, thus adding many species new to his Mona Island collection and much additional data about the island's entomological fauna. They are Messrs. Enrique Huyke and Antonio Ferrer Monge of Mayaguez, Puerto Rico, who spent several days on the island during the latter part of June and July respectively, and Mr. Harry A. Beatty of St. Croix, Virgin Islands, who stayed on the island from August

11 to August 31 and who collected at Sardinera and Uvero Beaches and all over the plateau.

ACKNOWLEDGEMENTS

Systematic Account of the Insects

Order Thysanura

Family LEPISMATIDAE

Five specimens of an undetermined lepismatid were taken under dead leaves near the cliff at Sardinera Beach, August 11–31, 1944.

Order COLLEMBOLA

Family ENTOMOBRYIDAE

Lepidocyrtinus sp.

Det. Grace E. Glance

Numerous specimens collected under stones, near Sardinera Beach, March 6, 1944.

Order ORTHOPTERA

Dr. B. B. Fulton, Department of Zoology and Entomology, North Carolina State College of Agriculture and Engineering, kindly determined or confirmed the writer's determinations of the Orthoptera obtained from Mona Island.

Family BLATTIDAE

Aglaopteryx devia Rehn

Reported from Mona Island by Rehn and Hebard 1927: 7–8 as *A. diaphana* Fabricius, which, according to Guerney 1937: 104, is a Cuban species that does not occur in the Puerto Rican region.

Blattella germanica Linnaeus

Wolcott 1941: 49 reports this species as a pest in houses, August 5, 1939 and March 30, 1940. Not observed by the writer during his trip to the island in April 2–7, 1944.

Symploce flagellata Hebard

Described by Hebard 1916: 367 from Desecheo and Mona Islands. Two of the paratypes were collected on Mona by F. E. Lutz on February 21 and 24, 1914. Rehn and Hebard 1927: 136 remark that this species "does not occur in the island of Puerto Rico itself".

Symploce bicolor Palisot de Beauvois

In houses, Sardinera Beach, March 29, 1940 (Wolcott 1941: 39).

Symploce bilabiata R. & H.

Determined A. B. Gurney.

Collected by Harry A. Beatty, Aug. 21, 1944

Pelmatosilpha coriacea Rehn

First recorded from Mona Island by Rehn and Hebard 1927: 149, who mention a male specimen taken on the island on February 24, 1914 by F. E. Lutz. Wolcott 1941: 40 records specimens taken under guava leaves and under the bark of a dead tree at Sardinera Beach, August 6, 1939.

The writer collected one specimen in the same locality under the bark of a dead tree, April 4, 1944.   He also has specimens taken at Uvero Beach on August 11–31, 1944.

Periplaneta americana Linnaeus
The writer has specimens taken in houses at Sardinera Beach, August 11–31, 1944, when the species was found rather abundant.

Periplaneta australasiae Fabricius
This species was found common in houses at Sardinera Beach on August 11–31, 1944.

Epilampra mona Rehn and Hebard
Rehn and Hebard 1927: 216–218 described this species from a single female specimen collected on *Tillandsia utriculata* L. at Sardinera Beach on February 24, 1914 by F. E. Lutz.   The writer collected one specimen under the bark of a dead tree at the same locality on April 4, 1944.

Pycnoscelus surinamensis Linnaeus
Rehn and Hebard 1927: 245 report specimens taken under wood at Sardinera Beach on February 21–26 by F. E. Lutz.   Wolcott 1941: 32 records specimens under stones at Camp Cofresí, August 7, 1939.   The writer has two specimens from Uvero Beach, August 11–31, 1944.

Family MANTIDAE

Callimantis antillarum Saussure
Wolcott 1941: 40 gives 3 records for this species from the island (Acc. Nos. 14–37; 53–40 and 252–40).   The writer collected numerous nymphs and adults at Sardinera Beach and Uvero Beach by sweeping over shrubbery and weeds, April 4–6, 1944.   He has other specimens taken July 20 and August 11–31, 1944.

Family PHASMIDAE

Lampomius bocki Brunner and Redtenbacher
Described from Mona by Brunner and Redtenbacher 1908: 357.

Aplopus sp.
Reported by Wolcott 1941: 40 (Acc. No. 16–37).

Family TETTIGIDAE

Paratettix frey-gessneri Bolivar
Numerous specimens were taken by the writer at Uvero Beach, April 1935.

Family LOCUSTIDAE (ACRYDIIDAE)

Sphingonotus haitiensis Saussure

Wolcott 1941: 40 reports numerous specimens from grass and weeds at the airport and Playa de Pájaros, August 9, 1939. The writer has specimens swept from vegetation at Sardinera and Uvero, April 5 and August 11–31, 1944.

Scyllina (Plectrotettix) gregarius Saussure

Reported by Wolcott 1936: 37 (Acc. No. 1318–13). The writer has specimens taken on weeds at Sardinera, Uvero and the plateau, April 4 and August 11–31, 1944.

Schistocerca americana Drury

Wolcott 1936: 37 reports this species for the first time from Mona (Acc. No. 1315–13). Later (1941: 40) he records it as abundant at Sardinera and on the plateau, feeding on grasses and trees, August 6, 1939 and March 30, 1940. The writer collected a nymph by sweeping over shrubs on the plateau, April 5, 1944.

Schistocerca columbina Thumberg

The only record of this species from the island is that given by Wolcott 1936: 37 (Acc. No. 1316–13).

Family TETTIGONIIDAE

Microcentrum triangulatum Brunner

Wolcott 1941: 40 records specimens taken at light at Sardinera Beach, August 8, 1939 and at the Lighthouse, April 1, 1940. The writer has specimens collected also at light at Sardinera Beach on March 3, July 22, and August 11–31, 1944.

Neoconocephalus triops Linnaeus

Two specimens taken on the plateau, August 11–31, 1944.

Conocephalus cinereus Thunberg

The writer collected numerous nymphs and adults on grass along the beach at Sardinera, April 4, 1944. He has other specimens from the same locality dated August 11–31, 1944.

Family GRYLLIDAE

Cycloptilum antillarum Redtenbacher

Numerous specimens were swept from vegetation at Sardinera and Uvero Beaches, April 1935, March 2 and August 11–31, 1944.

Cycloptilum sp.

Several specimens taken at Uvero Beach by sweeping on vegetation, April 4, July 21, and August 11–31, 1944.

Gryllus assimilis Fabricius

Uvero Beach, April 1935 and Sardinera Beach, August 11–31, 1944.

Cyrtoxipha gundlachi Saussure

Several specimens were swept from shrubbery at Uvero Beach, August 11–31, 1944.

Orocharis vaginalis Saussure

Several nymphs were swept from weeds at Uvero Beach on June 29, 1944. An adult was taken at Sardinera, August 11–31, 1944.

Oecanthus niveus De Geer

One specimen swept from shrubs, Sardinera Beach, June 29, 1944. Others at Uvero, August 11–31, 1944.

Amphiacusta caraibea Saussure

Wolcott 1941: 41 reports this cricket common in caves and houses, August 5–8, 1939. The writer noticed it very common in houses and under objects on the ground at Sardinera and Uvero Beaches, April 3–7, 1944. Specimens were taken on June 29, July 21, and August 11–31, 1944.

Family TRIDACTYLIDAE

Ellipes minuta Scudder

The writer collected one specimen at light, Sardinera Beach, April 7, 1944.

Order DERMAPTERA

Family LABIDURIDAE

Labidura riparia Pallas

Wolcott 1941: 39 reports this species at light at the Lighthouse, April 1, 1940.

Order ISOPTERA

The determinations of all the species of termites from Mona Island in the writer's collection were made by Professor Alfred Emerson, Department of Zoology, Chicago University, whose comments on the species are here included with his kind permission.

<center>Family KALOTERMITIDAE</center>

Kalotermes mona Banks

Banks 1919: 478 described this species from Mona Island from soldiers taken there on February 21, 1914.

Several soldiers and nymphs and a single dealate were collected by the writer on a dead branch of a living tree of *Melicocca bijuga* L. at Uvero Beach, April 5, 1944. Martorell also secured soldiers and a single alate on the same occasion. The writer's specimens were compared by Professor Emerson to a paratype in his collection and made topotypes by him. His determination is accompanied by the following remarks: "The collections mentioned above are all that are known of this species. So far no record has been obtained from any other locality than Mona Island. The species is quite distinct from all other described species, possibly being closer to *K. jouteli* Banks than to any other species. The imago caste has not been described."

Kalotermes snyderi Light

Wolcott 1941: 41 reports this species in "sanguinaria" tree, August 8, 1939. Martorell recorded it attacking the following trees: *Dipholis salicifolia, Metopium toxiferum, Elaphrium Simaruba, Amyris elemifera, Coccolobis uvifera, Canella Winteriana, Pithecollobium Unguis-cati* and *Gymanthes lucida* on April 1, 1940 (Acc. No. 296–40). Wolcott noted the species, April 5, 1944, on *Coccolobis laurifolia, Coccolobis uvifera, Conocarpus erecta, Rawolfia nitida* and *Metopium toxiferum* (Acc. No. 45–44).

Kalotermes incisus Silvestri

One soldier taken on July 21 and alates, soldiers and nymphs on August 11–31, 1944.

In giving his determination, Professor Emerson states: "I am not completely confident of the determination of this species. Silvestri described *K. incisus* from St. Jean, Venezuela, in 1903. The type is supposed to be in Copenhagen and has not been redescribed. The original description is not exact enough to be sure of the identity of the specimens before me. I have a fair number of alates and soldiers which are the same as the Mona Island specimens from St. Croix and Barbados. I have named these specimens *K. incisus* with some doubt. I also have a single alate from Caracas, Venezuela, which has a slightly smaller eye and may be a different species. I am thus unable to make accurate determinations until the type specimen has been examined or further collections have been made near the type locality".

Procryptotermes corniceps Snyder

Alates, one soldier, and nymphs, March 1; alates at light, Sardinera

Beach, April 7 and June 29; alates and nymphs from an old stump, Uvero Beach, August 11–31, 1944.

Professor Emerson's determination of this species is accompanied by the following remarks: "This species was described by Snyder in 1923 from 2 dealates and 2 soldiers from Boqueron-Salinas, Porto Rico. No further material has been collected since that time. I have one dealate and one soldier paratype in my collection with which I compared your specimens. The cotype soldier has a sharper ridge between the vertex and front which I assume to be within the expected variation but more extensive collections must be made before the significance of this variation can be determined. Snyder placed the species originally in *Glyptotermes* and later (1925) placed it in *Calcaritermes*. I have no hesitation in placing it in the genus *Procryptotermes* on the basis of both soldier and alate characters. The wings, which Snyder did not see, are of the *Cryptotermes* and *Procryptotermes* type and definitely different from the *Glyptotermes* and *Calcaritermes* type. The dentation of the imago mandible is also of the *Cryptotermes* and *Procryptotermes* type. The soldier is a more generalized type than found in *Cryptotermes*. The genus *Procryptotermes* was originally described from species from Madagascar and Aldabra. However, I have been determining both old species and new species as *Procryptotermes* for several years from various parts of the world including a number of New World species. One species very closely related to *P. croniceps* is found in Jamaica, but has not yet been described."

Order NEUROPTERA

Mr. Nathan Banks, Museum of Comparative Zoology, Harvard College, kindly determined all the specimens of Neuroptera from Mona Island in the author's collection.

Family CHRYSOPIDAE

Chrysopa thoracica Walker

This seems to be the most common chrysopid in the island. Martorell collected one specimen on the plateau, March 29, 1940 (Acc. No. 287–40), and the writer found it very abundantly at light at Sardinera Beach on April 7, 1944. Numerous specimens were also taken at light on June 29 and July 22 at Sardinera Beach.

Chrysopa transversa Walker

This species is known only from 2 specimens collected at light at Sardinera Beach on April 7, 1944.

Chrysopa damiensis Smith

A single specimen of this species was collected at light, Sardinera Beach, March 4, 1944.

Nodita haitiensis Smith

A specimen of this species collected on the island on April 1935, and determined by Mr. Nathan Banks, is deposited in the collection of the College of Agriculture at Mayaguez, Puerto Rico.

Family MYRMELEONIDAE

Psammoleon bistictus Hagen

Wolcott 1941: 47 reports this antlion common at light at Sardinera Beach on August 5–6, 1939 and April 1, 1940.

Psammoleon minora Banks

There are four specimens of this species in the author's collection labeled Sardinera Beach, July 29, 1944.

Myrmeleon insertus Hagen

The writer found this species rather common at light at Sardinera Beach on April 4–7, 1944. Numerous larvae, presumably of this species, were also found in loose dry sand in many places at the same locality.

Family ASCALAPHIDAE

Ululodes opposita Banks

Martorell collected specimens of this beautiful ascalaphid at Sardinera Beach on August 6, 1939 (Wolcott 1941: 48). The species is represented in the writer's collection by two specimens swept from shrubbery at Uvero Beach, August 11–31, 1944.

Order ODONATA

Family LIBELLULIDAE

Orthemis ferruginea Fabricius

Klots 1932: 7 records this species from Mona Island without date. Wolcott 1941: 47 reports the species common at Sardinera, March 30, 1940.

Erythrodiplax umbrata Linnaeus

Wolcott 1941: 47 reports specimens taken at Sardinera on August 6, 1939 and April 1, 1940.

Tramea abdominalis Rambur

Recorded from the island by Klots 1932: 7 without definite locality or date.

Lepthemis vesiculosa Fabricius

Several specimens observed at Sardinera on April 4–7, and August 11–31, 1944, but none collected.

Family COENAGRIONIDAE

Enallagna civille Hagen

Reported by Wolcott 1941: 47 from Sardinera, April 1, 1940.

Order MALLOPHAGA

Family PHILOPTERIDAE

Esthiopterum gracilicornis major Kellog

Wolcott 1941: 46 reports this species from man-o'-war bird, *Fregata magnificens rothschildi* Mathews (Acc. No. 388–39).

Order THYSANOPTERA

Family THRIPIDAE

Thrips tabaci Lindeman

This important and injurious species, commonly known as the onion thrips, is the only member of the order Thysanoptera so far recorded from Mona Island. Wolcott 1941: 49 reports it attacking onions planted at Camp Cofresí (Acc. No. 29–40) in 1940.

Order HOMOPTERA

Family MEMBRACIDAE

Paradarnoides danforthi, n. sp.   Figs. 1, 2, 3, and 4.

Head very broad and short, nearly concealed from above by pronotum, rugose, covered with abundant golden pubescence, fuscous, becoming paler on upper margin, lateral border at base of eyes, apical portion of post-clypeus, and outer margin of lorae; eyes large and prominent, testaceous, with a reddish rim near base; ocelli conspicuous, widely separated, closer to eyes than to each other, testaceous, flat in front; antennae placed in a large cavity directly below ocelli, testaceous, flagellum infuscated at base. Pronotum testaceous, rather closely and evenly ferrugineous-punctate, sides of metopidium, directly behind eyes, depressed, each with a large fuscous, rugged patch; median carina conspicuous throughout its entire length and decidedly more elevated on posterior process; lateral carinae running from shoulders to apex of posterior process, also conspicuously raised; shoulders set far back from eyes, obtusely prominent, width across them equal to that of head; posterior process gradually narrowed from shoulders to apex, with a slight sinuation near middle, tip distinctly surpassing abdomen. Tegmina hyaline, with base brownish and punctured, veins brown, outer ones with short, curved hairs. Underside fuscous, pubescent. Abdomen light yellow, tip infuscated; legs testa-

ceous, densely clothed with whitish long pubescence; anterior and middle femora and tibiae somewhat infuscated on anterior surface, tarsi fuscous.

Length including tegmina, 4.67 mm.; width across shoulders, 2.0 mm.

Type, male, Mona Island, June 29, 1944.

The genus *Paradarnoides* Fowler includes two other species, *severini* Fowler and *ignipes* Fowler, described from Guadeloupe in the Lesser Antilles (Fowler 1894: 423). The Mona Island species differs from these in its smaller size and different color.

The writer takes great pleasure in dedicating this species to the late Dr. Stuart T. Danforth, who was his close friend and constant teacher for many years.

Family BYTHOSCOPIDAE

Agallia albidula Uhler

Reported by Wolcott 1941: 50 on weeds at Sardinera, March 30, 1940. The writer has specimens swept from weeds at Sardinera Beach, April 4 and from *Pluchea purpurascens* at Uvero , April 5, 1944.

Family CICADELLIDAE

Hortensia similis Walker

A single specimen taken at light, Sardinera Beach, March 3, 1944.

Poeciloscarta histrio Fabricius

Wolcott 1941: 51 reports this species on castor bean at Rancho Grande, August 8, 1939, and abundant on grasses and at light (as *Cicadella sirena* Stål, a Mexican species), Sardinera Beach, March 30, 1940. The writer has specimens swept from weeds at Sardinera, March 1, and July 19, 1944.

Family JASSIDAE

Platymetopius loricatus Van Duzee

Specimens swept from shrubbery at Uvero, April 7, 1944.

Deltocephalus maculellus Osborn

One specimen swept from weeds, Uvero Beach, March 3, 1944.

Thamnotettix colonus Uhler

Specimens swept from weeds, Uvero Beach, March 3, 1944.

Thamnotettix cubanus Ball

A single specimen taken on weeds, Uvero Beach, March 3, 1944.

Chlorotettix tethys Van Duzee

Three specimens taken at light, Sardinera Beach, April 4–5, 1944.

Nesosteles guajanae DeLong

Specimens swept from weeds at Sardinera Beach, March 3, 1944.

Family EUPTERYGIDAE

Hybla maculata McAtee

Wolcott 1941: 53 reports this species abundant under the leaves of an unspecified plant at Sardinera Beach, March 31, 1940.

Family CIXIIDAE

Oliarus franciscanus Stål

Reported by Wolcott 1941: 53 (as *O. complectus* Ball) on weeds at Sardinera Beach, March 30, 1940.   The writer collected a single specimen by sweeping on weeds at Uvero, April 4, 1944.

Family ARAEOPIDAE

Liburnia furcifera Horváth

A single specimen taken at light, Sardinera Beach, March 3, 1944.

Family KINNARIDAE

Paraprosoptropis, n. gen.   Figs. 5, 6, 7, 8, and 9.

Head, across eyes, about three-fifths width of pronotum.   Vertex a little longer than wide, expanding basad; base about one and a half times width of vertex at narrowest point, angularly emarginate, with a conspicuous marginal carina; median and lateral carinae well-developed, running down to apex of frons.   Frons almost one and a half times longer than wide, base about half as wide as apex, sides gradually expanding for about four-fifths from base, then slightly converging towards apical margin, which is nearly straight; median and lateral carinae prominent.   Clypeus about as wide as long, base slightly but distinctly narrower than frons at widest part, sides converging acutely to apex; median carina very prominent, somewhat wider than that of frons, lateral carinae also well developed; surface slightly concave.   Antennae with basal segment very short, second segment stout, decidedly longer than broad; flagellum about $3\frac{1}{2}$ times longer than second segment.   Eyes deeply emarginate ventrally above antenna. Pronotum one and a half times longer than vertex, anterior margin sinuate behind eyes, then smoothly curving posteriorly, posterior border nearly straight, curving anteriorly at sides; tricarinate on disc, all 3 carinae well developed, lateral ones diverging towards apex; each lateral margin with a conspicuous carina running from eye to tegula.   Mesonotum very deeply and conspicuously depressed on disc, the concavity bordered on sides by nearly straight, strongly elevated submarginal carinae, which converge towards apex, meeting on a rounded tip; lateral margins depressed, evenly rounded posteriorly; tip of scutellum rounded.   Hind tibiae unarmed. Pregenital plate roughly trapezoidal, about as wide as long, basal border concavely curved, sides widening to about two-fifths from base, then gradually tapering posteriorly, angles obtusely rounded, surface with a shallow

depression on basal half and two smaller ones on apical half, at sides with short, scattered hairs.

Tegmina with sides expanding apically for about three-fifths of length, nearly symmetrically rounded at tip, length a little over two times greatest width; margin completely bordered, border widened below stigma, where it is also transversely rugose. Costal cell wide, feebly expanding toward apex; Sc and R joined to stigma; basal cell small, elongate; 7 apical cells; first trapezoidal, with inner side concavely curved, large; second trapezoidal; third smaller, triangular; fourth elongate, rectangular, medio-apical; fifth triangular, subequal to third; sixth and seventh pentagonal, the latter with upper inner side slightly concavely curved, and with one angle touching tip of clavus. Four small ante-apical cells, three with sides curved.

Anal segment of male bifid; aedaegus with 2 sclerotized rods and a pair of dorso-lateral spine-like processes; genital styles with a lateral eminence.

Genotype *Paraprosoptropis monensis*, n. sp.

This genus is closely related in many ways to *Prosoptropis* Uhler, from the Lesser Antilles. *Paraprosoptropis* differs from this and from all other described genera of West Indian Kinnaridae in several ways, principally in having the disc of the mesonotum depressed, and in possessing 4 ante-apical cells in the tegmina.

Paraprosoptropis monensis, n. sp.   Figs. 5, 6, 7, 8, and 9.

Vertex pale yellow, with posterior median region orange chrome; frons pale stramineous, a large orange chrome patch occupying most of upper area and gradually narrowing apically along sides of median carina; clypeus deep orange; eyes whitish, a small spot on upper inner region and emargination purplish; second joint of antennae pale yellow. Pronotum pale stramineous, a wide band of dark orange on basal half not reaching sides. Mesonotum dark orange on basal half, apical half very pale stramineous. Scutellum dark orange, tip pale stramineous. Tegmina hyaline, with 2 fuscous spots at base of clavus, first largest, second very small; 3 on costal area, middle one very large; an oblique fuscous band from base of seventh apical cell to base of first; a small fuscous spot between the first and second apical cells, on the border and another similarly placed between the sixth and seventh apical cells; and a large fuscous patch on the apical border; veins fuscous. Entire abdomen dark orange, edges of last 2 segments and anal segment very pale stramineous. Subgenital plate pale orange, narrowly infuscated on the edges and very faintly so on apical third. Legs pale stramineous, femora infuscated on the edges and very slightly so on apical half.

Anal segment of male very large, curving inwardly at apex which is forked; aedaegus short and broad, slightly curved upward; genital styles

irregularly sinuate on dorsal margin which is also setose, ending in a short, obtuse, upwardly curved hook; lateral eminence large, with a deep sinus on upper margin which is setose, terminating in a rounded point.

Length, female .94 mm.; tegmen 1.40 mm.; male. 88 mm.; tegmen 1.31 mm.

Holotype, female, Mona Island, August 11–31, 1944.

Allotype, male, Mona Island, April 7, 1944.

Family TROPIDUCHIDAE

Neurotmeta viridis Walker

Wolcott 1941: 53 records one specimen at light at Sardinera Beach, March 29, 1940.   The writer has numerous specimens collected from weeds at Uvero Beach, April 5 and June 29, 1944.

Family FLATIDAE

Petrusa marginata Brunnich.   Figs. 18 and 19.

Wolcott 1941: 53 records the dark form of this species (as *Ormenis marginata* Brunnich) on *Coccolobis uvifera, Coccolobis laurifolia,* and *Lantana,* and at light at Sardinera Beach, Camp Cofresí and Uvero Beach, August 5–7, 1939.   He gives the same records for the pale form which he reports as *O. pygmaea* Frabricius.   The writer has numerous specimens of both forms collected by sweeping on weeds at Uvero and Sardinera Beaches, March 4 and April 4–7, 1944.

The writer is following Fennah 1941: 193–195 and others, who regard *marginata* and *pygmaea* as a single species since both forms merge in coloration and have very similar genitalia.

Melormenis antillarum Kirkaldy

Recorded by Wolcott 1941: 53 (as *Ormensis quadripunctata* Fabricius) on *Coccolobis uvifera* at Playa de Pájaros, August 8, 1939.   The writer has specimens swept from weeds at Uvero, April 7, 1944.   The internal male genitalia are shown in figs. 14 and 17.

Flatoidinus pseudopunctatus, n. sp.   Figs. 10, 11, 12, 13, and 15.

Head much narrower than pronotum.   Vertex about two times as wide as length at middle, obtusely pointed anteriorly; lateral margins slightly arcuate; surface flat, with a T-shaped furrow on disc.   Frons one and a quarter times longer than greatest width; base as wide as apex, obtusely pointed; sides evenly arcuate, with a prominent, elevated marginal carina becoming obsolete apically; apical margin straight; surface gradually curved downward, slightly depressed on median apical half and conspicuously tumid on median basal portion.   Clypeus longer than width at base (1.4 to 1); sides converging apically and nearly straight; surface convex.

Pronotum slightly longer than vertex; a strongly elevated marginal carina on sides, behind eyes; hind border deeply and evenly emarginate; two large punctures on disc. Mesonotum very large; length at middle about equal to greatest width; front margin acutely rounded; hind margin gradually narrowing into an obtuse point, tip slightly raised; surface convex, disc slightly depressed. Tegmina long and broad, a little over two and a quarter times longer than wide; apical border broadly rounded; costal membrane almost reaching apex, about two times broader than costal cell, gradually narrowing posteriorly, with numerous transverse veins; 3 irregular sub-apical lines, not reaching costal margin. Front tibiae grooved on outer side, distally enlarged, with 3 ante-apical spines; middle and posterior tibiae trilateral.

General color yellowish-brown, underparts lighter; frons infuscated basally; mesonotum fuscous brown; tegmina slightly infuscated apically and along outer border of clavus; wings smoky-hyaline. Vertex with a narrow, longitudinally elongate fuscous spot on each side of median line; pronotum with the two fuscous punctures on disc and several fuscous spots behind eye; mesonotum and tegmina with scattered small fuscous markings.

Length to apex of tegmina, male, 8.43 mm.; female 8.23 mm.

Holotype, male, swept from weeds, Sardinera Beach, Mona Island, April 4, 1944.

Allotype, female, swept from weeds, Sardinera Beach, Mona Island, April 7, 1944.

Paratypes, 4 males, Mona Island, April 4–5, 1944.

The records given by Wolcott 1941 : 53 under *Flatoides punctatus* Walker, an entirely different flatid, should be referred to this species. He reported specimens taken at light, Camp Cofresí, August 6, 1939, on *Coccolobis uvifera*, *Coccolobis laurifolia*, and casuarina pines, August 6–7, 1939, and on "corcho" and "alelí" on the plateau, April 1, 1940.

Family ACANALONIIDAE

Acanalonia brevifrons Muir

One specimen swept from weeds on the plateau, June 29, 1944.

Acanalonia pumila Van Duzee

The writer found nymphs and adults of this species exceedingly abundant on *Mallotonia gnaphaloides* at Sardinera Beach, April 4, 1944.

Family ISSIDAE

Colpoptera maculata Dozier

Wolcott 1941 : 53 reports one specimen collected at Sardinera Beach, March 30, 1940.

Colpoptera flavifrons Osborn

The writer has numerous specimens swept from weeds at Sardinera and Uvero Beaches, March 4 and April 7, 1944. The male genitalia are shown in fig. 16.

Family CHERMIDAE

Ceropsylla sideroxyli Riley

Listed from Mona by Wolcott 1941: 54, on *Sideroxylon foetidissimum*.

Family APHIDIDAE

Aphis gossypii Glover

Wolcott 1941: 54 reports heavy infestations of watermelons by this aphid at Rancho Grande, March 31, 1940.

Macrosiphum ambrosiae Thomas

Martorell found this aphid on the leaves and branches of *Salvia splendens* which undoubtedly is an error for *Pluchea purpurascens* ("salvia" in Spanish) since that plant is not found in Mona. The author found the species on the tender leaves and stems of *Pluchea* at Sardinera and Uvero Beaches on April 5, 1944.

Family ALEYRODIDAE

Aleurothrixus floccosus Maskell

Wolcott found this species under the leaves of *Coccolobis uvifera* at Uvero Beach, April 7, 1944.

Family COCCIDAE

Icerya purchasi Maskell

Introduced into the island probably in the Australian pines planted by the Forestry Service at Sardinera Beach. First reported by Wolcott 1939: 33. Martorell observed a heavy infestation of the scale on casuarina pines and cultivated eggplants at Camp Cofresí and Rancho Grande Sardinera Beach, August 5, 1939 (Wolcott 1941: 56). The writer observed very few scales on the casuarina pines during his trip of April 4–7, 1944.

Ceroplastes sp.

A single, very large specimen, white and slightly pinkish, determined by Dr. G. N. Wolcott as a species of *Ceroplastes*, was collected by him on a leaf of *Coccolobis uvifera* at Uvero Beach, April 5, 1944.

Coccus viridis Green

Recorded by Wolcott 1941: 59 at Sardinera Beach on eggplants, *Terminalia Catappa*, *Coccolobis uvifera* and *C. laurifolia*, August 5–6, 1939.

The author found this species at the same locality on April 5, 1944 on the tender stems of *Rawolfia nitida*, attended by *Solenopsis geminata*.

Saissetia oleae Bernard

Wolcott 1941: 60 reports this scale insect, attended by *Solenopsis geminata*, infesting the leaves of *Terminalia Catappa* at Sardinera Beach, August 6, 1939. The author noted the species on the stems of wild cotton at Uvero Beach, April 5, 1944.

Pseudaulacaspis pentagona Targioni

Very scarce during the writer's trip to the island in April, 1944. The accidental occurrence of *Chilocorus cacti* on Mona is held responsible for the scarcity of this and other scale insects on the island by Wolcott 1944: 451–452.

Pinnaspis minor Maskell

Observed on cultivated eggplants at Rancho Grande, attended by *Solenopsis geminata*, August 8, (Acc. No. 119–39) and on mahogany at Camp Cofresí, August 6, 1939 (Acc. No. 120–39). Not very abundant but present on numerous plants, April 5, 1944 (Acc. No. 44–44).

Aspidiotus destructor Signoret

Wolcott 1941: 61 reports this scale abundant on *Barringtonia asiatica* and on cocoanuts at Sardinera Beach, August 5, and on the fruits and leaves of the first host at Playa de Pájaros, August 7, 1939. The writer observed the species heavily infesting the leaves of *Barringtonia* at Sardinera Beach, April 7, 1944, and also noted *Chilocorus cacti* feeding on them.

Pseudoparlatoria ostreata Cockerell

Wolcott 1941: 62 records this scale on the stems of wild papayas all over the island. During the writer's trip in April, 1944, the scale was very scarce, and was noted only on one occasion on which larvae and pupae of *Chilocorus* were also present.

Order HEMIPTERA

The writer is responsible for most of the determinations in this order.

Family NOTONECTIDAE

Buenoa femoralis Fieber

The only record of this species from Mona Island is that reported by Barber 1939: 421 based on specimens collected by F. E. Lutz on February 21–26, 1914.

Buenoa pallipes Fabricius

This is another species known only from the island by specimens collected by F. E. Lutz on February 21–26, 1914 and reported by Barber 1939 : 421.

Family CORIXIDAE

Trichocorixa verticalis Fieber

Det. R. I. Sailer

Found abundantly in several small ponds of stagnant water along Sardinera Beach, April 6 and August 11–31, 1944. This is a North American species which has not been found yet in Puerto Rico itself. It can be easily recognized from other species by its robust form and the large frontal depression of the males.

Family VELIIDAE

Microvelia robusta Uhler

First recorded from the island by Barber 1939: 411 from specimens collected by F. E. Lutz on February 21–26, 1914. Numerous apterous and winged specimens were taken from a small pool at the airfield on April 6, 1944.

Family GERRIDAE

Limnogonus franciscanus Stål

This is a common species in Puerto Rico and other West Indian islands. It was first reported from Mona Island by Barber 1939: 408 (February 21–26, 1914; F. E. Lutz). Wolcott 1941: 65 reports it as common in a pool and cistern at Sardinera Beach, August 5, 1939 and March 31, 1940. Several specimens were collected by the author in a pool at the same locality on April 4–7, 1944.

Family MIRIDAE

Pycnoderes quadrimaculatus Guerin

Martorell recorded this species on field beans at Sardinera Beach on August 6, 1939 (Acc. No. 171–39). A single specimen in the writer's collection was collected at light at Sardinera Beach on April 5, 1944.

Lygus apicalis Fieber

Wolcott 1941: 66 reports specimens collected at light (Acc. No. 377–39).

Polymerus cuneatus Distant

Several specimens were collected by the writer by sweeping on weeds at Uvero Beach, April 4, 1944.

Creontiades rubrinervis Stål

A single specimen swept from weeds at Uvero Beach, April 4, 1944, others taken at Sardinera on June 29, 1944.

Family ANTHOCORIDAE

Cardiastethus rugicollis Champion

Several specimens taken by sweeping on weeds, Sardinera Beach, April 4–6, 1944.

Asthenidea picta Uhler

Det. R. I. Sailer

Two specimens swept from herbage, Uvero Beach, August 11–31, 1944.

Xylocoris sordidus Reuter

Known from Mona Island only from specimens collected by F. E. Lutz on February 21–26, 1914 and reported by Barber 1939: 401.

Family CIMICIDAE

Cimex hemipterus Fabricius

The common tropical bedbug was observed by Martorell on beds at Camp Cofresí on March 28, 1940 and reported by Wolcott 1941: 67.

Family NABIDAE

Nabis capsicornis Germar

This cosmopolitan species is represented in the writer's collection of Mona Island insects by a single specimen taken by sweeping on herbage at Uvero Beach, August 11–31, 1944.

Family REDUVIIDAE

Zelus longipes Linnaeus

This species, although one of the most abundant reduviids in Puerto Rico, is rather uncommon in Mona Island. A single specimen in the collection of the College of Agriculture at Mayaguez, Puerto Rico is dated April 5, 1935. Wolcott 1941: 68 records one specimen feeding on *Cycloneda sanguinea* on August 5–7, 1939. There is another specimen in the author's collection, taken from weeds at Sardinera Beach, August 11–31, 1944.

Family PHYMATIDAE

Macrocephalus sp.

A single nymph collected on weeds at Sardinera, August 11–31, 1944, has been placed in this genus because the characters of the antennae and front legs.

Family TINGIDAE

Corythucha gossypii Fabricius

Recorded by Wolcott 1941: 71 on castor bean, August 8, 1939. The writer found it common at Sardinera, April 4, 1944, all stages infesting the undersides of the leaves of *Capparis flexuosa* and *Ricinus communis*.

Teleonemia prolixa Stål

Several specimens taken from weeds at Uvero Beach, April 5, and August 11–31, 1944, agree with specimens of *T. prolixa* from Puerto Rico, determined by H. G. Barber.

Family PYRRHOCORIDAE

Dysdercus andreae Linnaeus

Known only from specimens taken on February 21–26, 1914 by F. E. Lutz and reported by Barber 1939: 336.

Family LYGAEIDAE

Oncopeltus aulicus Fabricius

Recorded by Wolcott 1941: 73 on *Ricinus* at Rancho Grande, August 1939, and on blossoms of *Colubrina colubrina, Moringa moringa,* and *Pisonia albida* at the same locality on March 31, 1940. Not collected by the writer.

Oncopeltus semilimbatus Stål

Barber 1939: 336 lists a specimen in the American Museum of Natural History collected on the island on November 10, 1919. The writer has specimens swept from weeds at Uvero Beach on the following dates: April 1935; July 19 and August 11–31, 1944.

This species is very closely related to the above but, as pointed out by Barber 1939: 336, it can be readily separated from it by the difference in coloration. In *aulicus* the entire lateral margins of the pronotum and the tip of the scutellum are red; the premedian white discal spot of the membrane is reduced to a narrow white line; and the apical and outer margins of the membrane are narrowly white. In *semilimbatus* the black area of the pronotum is extended to the lateral margins so that they are not entirely red; the tip of the scutellum is black; the premedian white discal spot of the membrane is narrowly whitish.

Although recorded from other of the Greater Antilles and Mona Island, this species has not yet been reported from Puerto Rico itself.

Lygaeus (Craspeduchus) pulchellus Fabricius

This is the most common *Lygaeus* in the island. Barber 1939: 337 reports specimens collected on February 21–26, 1914 and on April 6, 1924.

The writer found it abundantly on several weeds on April 4–6, 1944. He has also numerous specimens dated March and August 11–31, 1944.

Lygaeus (Ochrimnus) collaris Fabricius

This species is not common in the island. Wolcott 1941: 72 records it on flowers of *Pisonia albida* on April 2, 1940. The writer collected a single specimen by sweeping on weeds, April 4, 1944.

Lygaeus (Melanocoryphus) albonotatus Barber

This small and interesting species was described by Barber 1923: 2 from a single specimen collected on February 24, 1914. This unique specimen is deposited in the American Museum of Natural History. The writer was not able to collect this species during his trips to the island in spite of very diligent search for it.

Nysius ericae Schilling

This widely distributed species was first collected on the island on February 21–26, 1914 by H. E. Crampton. This record is reported by Barber 1939: 342. The writer has specimens dated April 1935 and July 20, 1944.

Nysius inaequalis Uhler

Barber 1939: 341 reports specimens collected by Crampton on February 22, 1914. The writer has a single specimen collected by him from herbage on April 1935.

Nysius strigosus Uhler

Known only from specimens collected on the island on February 21–26, 1914 by F. E. Lutz and reported by Barber 1939: 342.

Ischnorhynchus championi Distant

Collected by H. E. Crampton, February 15, 1914, as reported by Barber 1939: 344. The writer found it exceedingly abundant at Sardinera and Uvero Beaches, April 4–7, 1944, where he obtained numerous specimens by sweeping on shrubbery and low herbage.

Blissus leucopterus Say

First recorded from Mona by Barber 1939: 345 from specimens taken by Crampton on February 22, 1914. Wolcott 1941: 73 lists this species from grasses at Rancho Grande, August 7–8, 1939.

Geocoris thoracicus Fieber

One specimen secured by sweeping on weeds on the plateau, July 20, 1944. A second specimen was taken in the same way at Uvero Beach August 11–31, 1944.

Pachygrontha parvula Barber

This species was described by Barber 1923 : 4 from a single male collected on the island on February, 1914. This specimen is in the collection of the American Museum of Natural History.

Paromius longulus Dallas

Known from Mona only by the specimens collected there by F. E. Lutz on February 21–26, 1914 and reported by Barber 1939 : 350.

Pachybrachius vinctus Say

Collected by F. E. Lutz on February 21–26, 1914 (Barber 1939 : 353). Three specimens in the author's collection were taken on weeds at Sardinera Beach, August 11–31, 1944.

Pachybrachius scutellatus Dallas

The following specimens in the author's collection were secured from Mona Island: April 1935 (Det. H. G. Barber); April 7, 1944 (at light); August 11–31, 1944 (swept from weeds).

Heraeus guttatus Dallas

Two specimens were collected at light, Sardinera Beach, April 5, 1944. This species was previously known from Puerto Rico only by a single specimen collected at Isabela, April 24, 1930.

Ozophora atropicta Barber

Numerous specimens taken at light, Sardinera Beach, April 4–7, 1944.

Ozophora octomaculata, n. sp. Fig. 20.

Head black, with numerous short, appressed, whitish hairs on anterior dorsal surface, dorsal median area and undersurface; with 10 long setae between eyes and several shorter ones at tip of tylus; tylus ferrugineous; eyes with a reddish tinge principally around base; ocelli bright red; antennae with basal segment ferrugineous, segments II and III stramineous, and terminal one brownish; rostrum ferrugineous. Pronotum black, with 8 conspicuous, yellowish-orange, calloused spots placed as follows: 2 small, transversely elongated ones on collar; 4 larger and rounded ones equidistantly placed on disc of posterior lobe, lower ones reaching hind border of pronotum; and 2 largest ones on humerals. Scutellum black, with 2 submarginal inconspicuous, yellowish spots on disc parallel to sides; tip white. Hemelytra fuscous, with commissure, narrow costal margin, and radius yellowish-white, and with claval suture, claval vein and media, brownish-white; membrane smoky brown, with veins, inner basal angle, and broad apical margin whitish. Underparts black; venter with castan-

eous tinge and with numerous long hairs principally on hind border of last segments. Femora ferrugineous; tibiae and tarsi stramineous.

Head wider than long (.96 x .86 mm.). Length of antennal segments as follows: I, .53; II, 1.53; III, 1.10; IV, 1.60 mm. Rostrum extending to hind border of first visible abdominal segment; length of segments as follows: I, .90; II, 1.03; III, .73; and IV, .43 mm. Pronotum about one-third shorter than wide (1.10 x 1.56 mm.); anterior lobe slightly shorter than posterior lobe; disc of anterior lobe smooth except for a few very fine and inconspicuous punctures on median region and on sides; posterior lobe deeply and rather closely punctate except for calloused spots on humeral angles and on disc. Scutellum a little longer than wide (.83 x .76 mm.), much shorter than pronotum but distinctly longer than commissure; disc shallowly but distinctly sunken, rather deeply and closely punctate; region surrounding submarginal calloused spots impunctate; lateral submargins depressed, with 2 rows of close punctures. Hemelytra with costal margin very slightly concavely sinuate about one-third away from base; clavus with 6 rows of close punctures; corium also rather closely punctate along the subcostal area and along the borders of the veins. Anterior femora swollen, with 5 spines below on apical half. Length 5.60 mm.; width across humerals 1.56 mm.

Holotype, male, collected at light, Sardinera Beach, Mona Island, April 4, 1944.

Paratypes, 7 males and 2 females with same data as type; 6 males and 2 females, some data as type but April 5; and 2 males and 2 females, same data as type but April 7.

This species ressembles *O. subimpicta* Barber and *O. quinquemaculata* Barber in size and shape but it can be readily distinguished from them by the 8 conspicuous yellowish-orange spots on the pronotum which give the species its name.

Paragonatas divergens Distant

A single specimen taken at light, Sardinera Beach, March 5, 1944.

Family NEIDIDAE

Jalysus reductus Barber

This small stilt bug was described by Barber 1939: 331 from numerous specimens from the West Indies and Central America. The type, a male deposited in the American Museum of Natural History, was collected on Mona Island, February 21–26, 1914, by F. E. Lutz.

Family COREIDAE

Phthia picta Drury

Collected by Martorell on eggplants at Rancho Grande, August 8, 1939 (Wolcott 1941: 75).

Catorhintha guttula Fabricius

This is a rather common species in Mona. F. E. Lutz collected it on February 21–26, 1914 (Barber 1939: 318). In the author's collection there are numerous specimens from the island dated as follows: April, 1935; April 4–7, July 19, and August 11–31, 1944. Martorell recorded the species as abundant on corn leaves at Camp Cofresí, August 7–8, 1939 (Acc. No. 100–39).

Sphictyrtus whitei Guérin-Méneville

This beautiful large coreid, known locally as "avispilla" because of the wasp-like flight and buzzing sound produced when disturbed, is extremely abundant on Mona Island. Barber 1939: 321 lists specimens taken there on December 20, 1913; February 21–26, 1914; September 10, 1919; and March 10, 1926. Sein noted adults feeding on corn in 1926 but did not succeed in finding the nymphs or eggs (Wolcott 1936: 173). Wolcott 1941: 76 records the species as common on August 5–9, 1939 and again in 1940, remarking: "Adults in swarms clinging to lower branches of trees in shade of cliff, no apparent preference as to kind of tree, and not feeding. At top of cliff, adults on tender leaves of *Coccolobis laurifolia*, possibly feeding". The writer did not observe the species during his trip to the island in April, 1935, but found it abundantly on coconut palms and other plants at Sardinera Beach on April 4–7, 1944. He also observed adults feeding on the flowers of *Colubrina colubrina* on that occasion.

The species was described from Cuba in 1857 and has also been reported from San Salvador, Bahamas. It is not known from the Puerto Rican mainland.

Leptocorisa filiformis Fabricius

Wolcott 1941: 76 lists one specimen taken on weeds at Sardinera, August 8, 1939.

Hyalmenus longispinus Stål

Barber 1939: 323 records this species from Mona based on specimens collected by F. E. Lutz on February 21–26, 1914.

Corizus (Liorhyssus) hyalinus Fabricius

This common and widely spread species was first recorded from the island by Barber 1939: 327, who reports specimens taken on February 21–26, 1914 by F. E. Lutz. The writer has numerous specimens swept from weeds at Sardinera and Uvero Beaches, August 11–31, 1944.

Corizus sidae Fabricius

F. E. Lutz secured specimens on February 21, 1914, as reported by Barber 1939: 327. The writer collected several specimens (Det. H. G. Barber) in April, 1935.

Jadera haematoloma Herrich-Schaeffer

Numerous nymphs and adults of this species were collected by the author at Uvero Beach on April 5, 1944 under dead leaves and on the dry culms of guinea grass. This is a continental species which, according to Blatchley 1926: 286, was previously known only from Cuba in the West Indies. The species is readily distinguished from other species of *Jadera* in the West Indian region by the red and black color and conspicuous median carina on the pronotum.

Family PENTATOMIDAE

Mormidea angustata Stål

Barber 1939: 288 reports specimens collected by F. E. Lutz on February, 1914. This Mexican species is also known from the Isle of Pines and the Puerto Rican mainland in the West Indies.

Thyanta perditor Fabricius

Barber 1939: 292 reports specimens collected by F. E. Lutz on February 21–26, 1914. The author collected a single specimen on the island in April, 1935.

Thyanta antiguensis Westwood

A single specimen collected by sweeping on weeds, Sardinera Beach, August 11–31, 1944.

Nezara viridula Linnaeus

Wolcott 1936: 77 lists this species from Mona (Acc. No. 1319–13). Martorell collected one specimen at light at Camp Cofresí, August 7, 1939, and several others from weeds on the plateau, March 30, 1940 (Wolcott 1941: 78). The writer has numerous specimens from different localities on the island with the following dates: April, 1935; April 4, June 29, July 19 and 20, and August 11–31, 1944.

Acrosternum marginatum Palisot de Beauvois

Wolcott 1941: 78 reports specimens at light, Camp Cofresí and on weeds at Rancho Grande, August 7–8, 1939. The writer collected specimens on weeds at Sardinera Beach, April 5, 1944.

Arvelius albopunctatus DeGeer

Martorell collected a single specimen on eggplants at Rancho Grande, August 7, 1939 (Wolcott 1941: 78).

Brepholoxa rotundifrons Barber

A single specimen collected on weeds on the plateau, August 11–31, 1944.

Podissus sagitta Fabricius

One specimen collected on weeds, Sardinera Beach, August 11–31, 1944.

Pachycoris fabricii Linneaus

The author collected specimens on Mona, April, 1935. He has other specimens collected by sweeping on weeds on the plateau, March 5, 1944.

Diolcus irroratus Fabricius

Specimens collected on weeds, April, 1935 and April 5 and July 20, 1944.

Order COLEOPTERA

Family CARABIDAE

Tachys ensenadae Mutchler

Det. J. M. Valentine

At light, Sardinera Beach, April 5, 1944; under dead leaves near cliff, Uvero Beach, August 11–31, 1944.

Tachys sp.

Det. J. M. Valentine

A single specimen under trash near cliff, Uvero Beach, August 11–31, 1944.

Tetragonoderus sp.

Det. J. M. Valentine

Numerous specimens taken on the ground, Sardinera Beach, August 11–31, 1944.

Selenophorus sinuatus Gyllenhall

Det. P. J. Darlington, Jr.

Several specimens, April, 1935 and August 11–31, 1944.

Seleneophorus alternans Dejean

Det. J. M. Valentine

Many specimens under stones and dead leaves near the cliff, Sardinera Beach, August 11–31, 1944.

Selenophorus sp.

A single specimen collected at light, Sardinera Beach, April 1, 1940 (Acc. No. 289–40).

Apenes sp.

A single specimen at light, Sardinera Beach, March 7, 1944.

Family DYTISCIDAE

Copelatus angustatus Chevrolat

At light, Sardinera Beach, June 29 and July 22, 1944.   Many specimens from a small pool near the airfield, August 11–31, 1944.

Rhantus calidus Fabricius

Det. L. L. Buchanan

One specimen at light, Sardinera Beach, August 11–31, 1944.

Thermonectes circumscripta Latreille

Specimens taken in a small pool near the airfield, August 11–31, 1944.

Family HYDROPHILIDAE

Enochrus nebulosus Say

Specimens at light, Sardinera Beach, April 6 and June 29, 1944.

Berosus interstitialis Knisch

A single specimen at light, Sardinera Beach, April 6, 1944; others from a small pool near the airfield, August 11–31, 1944.

Hydrophilus ater Olivier subsp. **intermedius** DuVal

One specimen at light, Sardinera Beach, June 29, 1944.

Tropisternus lateralis Fabricius

Several specimens from a small pool near airfield, August 11–31, 1944.

Cercyon sp.

A single specimen at light, Sardinera Beach, April 4, 1944.

Family HISTERIDAE

Omalodes klugi Marseul

One specimen at Sardinera Beach, June 29, 1944.

Saprinus sp.

Det. H. S. Barber

Many specimens under a dead bird, Sardinera Beach, April 6–7, 1944.

Family STAPHYLINIDAE

Oxytelus incisus Mots.

Det. R. E. Blackwelder

Many specimens collected under a dead bird, Sardinera Beach, April 6–7, 1944.

Lithocharis sp.
Det. R. E. Blackwelder
Many specimens under trash, Sardinera Beach, June 29 and August 11–31, 1944.

Philonthus havaniensis Laporte
Det. R. E. Blackwelder
Numerous specimens under trash, Sardinera Beach, June 29, 1944.

Philonthus ventralis Gravenhorst
Det. R. E. Blackwelder
Several specimens under a dead bird, Sardinera Beach, April 4, 1944.

Cafius bistriatus Erichson
Listed from Mona by Blackwelder 1944 : 135. One specimen (Det. R. E. Blackwelder) collected under trash, Sardinera Beach, August 11–31, 1944.

Cafius subtilis Cameron
Det. R. E. Blackwelder
Many specimens under trash, Sardinera Beach, August 11–31, 1944.

Xantholinus beattyi Blackwelder
Det. R. E. Blackwelder
Several specimens under a dead bird, Sardinera Beach, April 4, 1944.

Family CANTHARIDAE

Tytthonyx cavicornis Leng and Mutchler
Described by Leng and Mutchler 1922 : 489 from a single specimen taken by F. E. Lutz on February 26, 1914. The type is in the collection of the American Museum of Natural History. The writer has 3 specimens swept from shrubs on the plateau, April 5 and June 29, 1944.

Tylocerus barberi Leng and Mutchler
One specimen at light, Sardinera Beach, March 3, 1944.

Family MELYRIDAE

Melyrodes sp.
Det. H. S. Barber
One specimen, Sardinera Beach, August 11–31, 1944.

Family CORYNETIDAE

Necrobia rufipes DeGeer
Numerous specimens taken on decaying fish at Sardinera Beach, March 6, 1944. Others on a dead goat on the plateau, April 7, 1944.

Family ELATERIDAE

Adelocera rubida Schwarz

Described by Schwarz 1902:193.

Conoderus figuralis Candeze

Det. M. C. Lane

Three specimens swept from weeds, Sardinera Beach, April 7, 1944.

Conoderus sericatus Candeze

Det. M. C. Lane

Numerous specimens at light, Sardinera Beach, March 1–5, April 4–7, and July 20, 1944.

Drasterius elegans Fabricius

Det. W. S. Fisher

At light, Camp Cofresí, March 31, 1940 (Acc. No. 280–40).

Dicrepidius ramicornis Palisot de Beauvois

Wolcott 1941: 88 lists one specimen taken on *Ricinus* at Camp Cofresí, August 7, 1939.

Esthesopus poedicus Candeze

Det. J. M. Valentine

One specimen swept from vegetation, Sardinera Beach, August 11–31, 1944.

Family BUPRESTIDAE

Acmaeodera gundlachi Fisher

Wolcott 1941: 88 reports one specimen resting on weeds at Camp Cofresí, Sardinera Beach, August 7, 1939. The author has several specimens collected on flowers and on weeds at Uvero Beach, April 7, May 24, and August 11–31, 1944.

Polycesta thomae Chevrolat

Martorell and the writer collected larvae, pupae and adults from the dead stems of casuarina pines at Sardinera Beach, April 6, 1944.

Chrysobothris megacephala Castelnau and Gory

One specimen resting on a branch of *Solanum verbascifolium* at Uvero Beach, April 7, 1944.

Micrasta oakleyi Fisher

Two specimens (Det. W. S. Fisher), April, 1935. One specimen on castor bean leaf at Rancho Grande, August 8, 1939 (Wolcott 1941: 89).

Family DERMESTIDAE

Dermestes canina Germar

Wolcott 1941: 89 reports larvae and adults in the shell of a dead turtle and on goat hides at the Lighthouse, April 1, 1940. Numerous larvae and adults were collected by the author on dead fish at Sardinera Beach, April 4–6, 1944.

Family OSTOMATIDAE

Tenebroides mauritanica Linnaeus

Wolcott 1941: 89 records one specimen at light, Camp Cofresí, August 7, 1939. The writer has in his collection another specimen also taken at light on the same locality, March 4, 1944.

Family NITIDULIDAE

Carpophilus sp. (**dimidiatus** Fabricius?)

Det. E. A. Chapin

Numerous specimens collected at Sardinera Beach, August 11–31, 1944.

Haptoncus luteolus Erichson

Recorded by Wolcott 1941: 90 at light (Acc. No. 379–39). Numerous specimens (Det. E. A. Chapin) taken at Sardinera Beach, August 11–31, 1944.

Stelidota strigosa Gyllenhall

Det. E. A. Chapin

One specimen taken at Sardinera Beach, August 11–31, 1944.

Family MONOTOMIDAE

Europs sp.

Det. W. S. Fisher

Several specimens swept from vegetation, Sardinera Beach, August 11–31, 1944.

Family CUCUJIDAE

Ahasverus (Cathartus) advena Waltl

One specimen under the bark of a dead tree, Camp Cofresí, August 9, 1939 (Wolcott 1941: 90).

Ahasverus rectus LeConte

Det. W. S. Fisher

Many specimens, Sardinera Beach, August 11–31, 1944.

Family EROTYLIDAE

Mycotretus sp.
Det. W. S. Fisher
Martorell obtained specimens at light at the Lighthouse, April 1, 1940.

Family CRYPTOPHAGIDAE

Loberus sp.
Det. W. S. Fisher
Martorell observed this species on watermelons at Rancho Grande, March 30, 1940.   The writer collected numerous specimens by sweeping on weeds at Sardinera and Uvero Beaches, April 4 and August 11–31, 1944.

Family PHALACRIDAE

Acylomus sp.
Reported by Wolcott 1941: 91 as taken at light (Acc. No. 379–39).

Family MYCETOPHAGIDAE

Typhaea stercorea Linnaeus
Det. W. S. Fisher
Specimens at Sardinera Beach, June 29, 1944.

Family COCCINELLIDAE

Scymnus roseicollis Mulsant
On castor bean at Rancho Grande, August 8, 1939 (Wolcott 1941: 92). One specimen swept from vegetation, Uvero Beach, August 11–31, 1944.

Scymnus floralis Fabricius
Swept from herbage at Sardinera and Uvero Beaches; also on the plateau, March 1, June 29, and August 11–31, 1944.

Scymnus sp.
Two specimens taken on April, 1935.

Rodolia cardinalis Mulsant
Introduced to control *Icerya purchasi*.   Martorell reports empty cocoons on casuarinas but not a single adult seen at Camp Cofresí, August 5, 1939 (Acc. No. 125–39).   On March 29, 1940 he found it very abundant and well established (Acc. No. 245–40).   Wolcott found empty pupal skins abundant and a few live ones on April 5, 1944.   He remarks that control of the scale was 99 per cent effective but always enough surviving to maintain both scales and predators (Acc. No. 32–44).

Psyllobora nana Mulsant

Specimens taken on weeds, April, 1935; March 3, and April 4, 1944. Also at light, April 5, 1944.

Psyllobora lineola Fabricius

Many specimens taken on weeds, April, 1935.

Cycloneda sanguinea Linnaeus

Numerous specimens swept from vegetation, April, 1935 and March 4, 1944. Wolcott 1941: 92 reports this species common on weeds.

Chilocorus cacti Linnaeus

This ladybeetle, not previously known from Mona, was first observed there by Wolcott, Martorell and the writer on April 4–7, 1944, when several adults and pupae were noted at Sardinera and Uvero Beaches on previously scale-infested wild papayas and a single plant of *Barringtonia asiatica*. Wolcott and Martorell 1944: 451–452 believe that the species probably reached Mona by its own initiative from the Puerto Rican mainland, where it was introduced from Cuba and Texas to control scale insects. Additional specimens were also obtained from the same localities on August 11–31, 1944.

Family OEDEMERIDAE

Copidita (Asclera) litoris Wolcott

A single specimen taken at light, Sardinera Beach, March 4, 1944, and determined by Dr. J. M. Valentine as *Copidita (Asclera)* sp., agrees perfectly with the species described by Wolcott 1936: 206 as *Oxacis litoris* from specimens collected on the beach of the north coast of Puerto Rico.

This rather small, very slender and elongate beetle has the prothorax dark iridescent green; the elytra are purplish, with the inner margin somewhat yellowish, and the legs dull yellowish orange.

Copidita (Asclera) sp. a.

Det. J. M. Valentine

A single specimen at light, Sardinera Beach, July 20, 1944. The entire beetle is dull violet-blue in color.

Copidita (Asclera) sp. b.

Det. J. M. Valentine

One specimen at light, Sardinera Beach, June 29, 1944; others swept from weeds at the plateau, August 11–31, 1944. This is a purplish species with the prothorax brownish red, the eyes and antennae nearly black and the elytra with the outer margins and a median longitudinal ridge whitish.

The beetles are identical with specimens from Desecheo Island (about 30 miles northeast of Mona) in the collection of the College of Agriculture, Mayaguez, Puerto Rico, determined as *Ditylus* sp. nov. by Dr. A. J. Mutchler.

Oxacis geniculata Chevrolat

Det. J. M. Valentine

Numerous specimens taken at light, Sardinera Beach, March 4–6 and April 5–6, 1944. This is the second most abundant and the largest oedemerid on the island. The head, except for the black eyes and infuscated basal segment of the antennae, and the entire prothorax are yellowish. The abdomen and elytra, except the outer and inner margins, are greyish-blue. The legs are light yellow, with the apical half of the femora strongly infuscated.

Alloxacis sp. a.

Det. J. M. Valentine

Two specimens at light, Sardinera Beach, June 29, 1944. This is a small, slender species of a deep metallic blue color.

Alloxacis sp. b.

Det. J. M. Valentine

This is the most abundant member of the family in Mona Island. The author has numerous specimens dated April, 1935; March 4–6, and April 4–6, 1944 (taken at light, Sardinera Beach). Adults were also observed by him feeding on the pollen of the flowers of *Colubrina colubrina* at the same locality on April 4. The records given by Wolcott 1941: 86 for *Oxacis litoris* probably refer to this species.

This is a very dull and dark blue species, the eyes are black and the antennae, except for the two basal segments, are reddish brown.

Alloxacis sp. c.

Det. J M. Valentine

Specimens at light, Sardinera Beach, March 4, 1944.

In this species, the head, except for the black eyes, the prothorax and scutellum are yellow. The antennae are infuscated on the basal half. The elytra are dull greyish-blue, each with 2 inconspicuous elevated lines. The legs are yellowish, with the apical portion of the femora somewhat infuscated.

Sessinia vittata Fabricius

Det. J. M. Valentine

One specimen swept from weeds at Sardinera Beach, April 7, 1944. Wolcott 1941: 86 reports the species (as *Ananca vittata* Fabricius) very

abundant at light and on sea-grape at Camp Cofresí and Playa de Pájaros, August 7–8, 1939 and March 30, 1940.

Family MORDELLIDAE

Mordellistena sp. a.
Three specimens swept from weeds and low shrubs on the plateau, June 29, 1944.

Mordellistena sp. b.
A single specimen from weeds at Sardinera Beach, August 11–31, 1944.

Family ANTHICIDAE

Anthicus (Omonadus) floralis Linnaeus
Det. J. M. Valentine
Two specimens at light, Sardinera Beach, June 29, 1944.

Family ALLECULIDAE

Hymenorus sp.
Wolcott 1941: 93 reports specimens common at light, Camp Cofresí, and on weeds at the airfield, August 5–6, 1939.   The writer has numerous specimens also taken at light at Sardinera Beach, March 4, 1944, and on weeds at Uvero and Sardinera Beaches, April 4, June 29, and July 20, 1944.

Family TENEBRIONIDAE

Opatrinus pullus Sahlberg
Reported by Wolcott 1941: 93 at light, Camp Cofresí, August 5, 1939.

Trientoma varvasi Solier
Specimens swept from vegetation, Uvero Beach, July 21 and August 11–31, 1944.

Blapstinus punctatus Fabricius
Wolcott 1941: 93 reports specimens at light, Camp Cofresí, August 8, 1939.   The writer has specimens also taken at light at the same locality on March 7, 1944.

Blapstinus sp.
Det. R. E. Blackwelder
Many specimens taken under trash, Sardinera Beach, March 4, April 5, and August 11–31, 1944.

Phaleria angustata Chevrolat
Det. R. E. Blackwelder
Numerous specimens taken under trash, Sardinera Beach, August 11–31, 1944.

Phaleria variabilis Quedenfeldt

Wolcott 1941: 93 reports specimens at light, Camp Cofresí, August 8, 1939. The writer collected specimens also at light at the same place on April 4, 1944.

Crypticus sp.

Det. R. E. Blackwelder

One specimen at Sardinera Beach, August 11–31, 1944.

Diaperis hydni Fabricius

Martorell and the writer collected one specimen at light, Sardinera Beach, April 7, 1944.

Tribolium castaneum Herbst

Two specimens taken at light, Sardinera Beach, April 7, 1944.

Doliema pallida Say

Wolcott 1941: 94 reports specimens under the bark of *Canella Winteriana* at Rancho Grande, August 8, 1939. The author has specimens taken under the bark of a dead tree at Sardinera Beach, June 29, 1944.

Family CISIDAE

Ceracis sp.

Det. W. S. Fisher

Abundant on fungi at Sardinera Beach, August 11–31, 1944.

Family ANOBIIDAE

Lasioderma serricorne Fabricius

One specimen at light, Sardinera Beach, April 7, 1944.

Family BOSTRICHIDAE

Tetrapriocera longicornis Olivier

Det. W. S. Fisher

One specimen at light, Sardinera Beach, April 7, 1944.

Heterarthron gonagrum Fabricius

First reported from Mona by Leng and Mutchler 1914: 453. The writer has several specimens taken on April, 1935, boring in casuarinas.

Xylomeria torquata Fabricius

Wolcott 1941: 95 reports numerous specimens at light, Camp Cofresí, August 6, 1939.

Family TROGIDAE

Trox suberosus Fabricius

One specimen at light, Sardinera Beach, August 11–31, 1944.

Family SCARABAEIDAE

Aphodius cuniculus Chevrolat

Common at light, Sardinera Beach, March 3–4 and April 4–7, 1944. Also on fresh cow dung at the same locality, April 5, 1944.

Ataenius darlingtoni Hinton

One specimen at light, Sardinera Beach, March 5, 1944.

Ataenius beattyi Chapin

Several specimens taken at Sardinera Beach, August 11–31, 1944.

Ataenius miamii Cartwright

Det. E. A. Chapin

Several specimens collected under trash near the cliff, Sardinera Beach, August 11–31, 1944.

Cnemarachis monana Moser

Described from Mona by Moser 1921: 181. Wolcott 1941: 97 reports adults at light, August 6, 1939 and March 31, 1940. The writer has specimens taken at light, Sardinera Beach, March 2, April 4–5, and June 29, 1944. He collected numerous larvae and pupae at Uvero Beach, April 5, 1944, under the roots of guinea grass.

Ligyrus tumulosus Burmeister

Wolcott 1941: 98 reports many adults collected at light at Camp Cofresí, August 5–7, 1939 and March 29–30, 1940. The author has numerous adults collected also at light at the same place on March 4, April 4–7, June 29 and August 11–31, 1944.

Strataegus barbigerus Chapin

Two specimens, a male and a female, in rotten stump of *Metopium toxiferum* on the plateau above Sardinera, August 7, 1939 (Wolcott 1941: 98). One female specimen at light, Sardinera Beach, August 11–31, 1944.

Family CERAMBYCIDAE

Stenodontes bituberculatus Palisot de Beauvois

Wolcott 1941: 98 reports an adult taken in an old tree stump at Rancho Grande, August 7, 1939.

Methia necydalea Fabricius

Wolcott 1941: 98 reports specimens collected at light, Camp Cofresí, August 6–7, 1939 and at the Lighthouse, April 1, 1940.

Eburia quadrimaculata Linnaeus

Reported by Wolcott 1941: 98 as very abundant at light, Camp Cofresí, August 5–6, 1939. The author has two specimens collected at light at Sardinera Beach, April 4 and June 29, 1944.

Elaphidion conspersum Newman

Two specimens at light, Sardinera Beach, April 4 and 7, 1944.

Elaphidion insulare Newman

Reported by Wolcott 1941: 98 (Acc. No. 13–37) without definite date or locality.

Elaphidion irroratum Linnaeus

Wolcott 1941: 99 reports this species common at light, Camp Cofresí, August 5–6, 1939. The writer has one specimen taken at light, Sardinera Beach, August 11–31, 1944.

Elaphidion spinicorne Drury

Wolcott 1941: 99 reports specimens at light, Camp Cofresí, August 5–6, 1939 and April 1, 1940.

Merostenus attenuatus Chevrolat

One specimen at light, Sardinera Beach, March 30, 1940 (Wolcott 1941: 99).

Cylindera flava Fabricius

Wolcott 1941: 99 reports one specimen at light, Camp Cofresí, August 8, 1939. The writer has another specimen also taken at light at the same locality, August 11–31, 1944.

Lepturges guadeloupensis Fleutiaux and Sallé

Det. W. S. Fisher

One specimen at light, Sardinera Beach, August 11–31, 1944.

Family CHRYSOMELIDAE

Pachybrachys mendicus Weise

Several specimens swept from herbage on the plateau above Uvero Beach, August 11–31, 1944.

Cryptocephalus multiguttatus Suffrian

The writer has numerous specimens in his collection dated April, 1935; March 3, June 29, July 20, and August 11–31, 1944, swept from weeds at Sardinera and Uvero Beaches and also on the plateau.

Nodonota wolcotti Bryant

Wolcott 1941: 100 reports this species on weeds. The writer has specimens swept from weeds at Sardinera Beach, March 6 and April 7, 1944.

Hermaeophaga cylindrica Weise

Reported by Wolcott 1941: 101 (Acc. No. 47–40).

Longitarus sp.

  Det. H. S. Barber

  Specimens taken at light, Sardinera Beach, April 7, 1944.

Aphthona compressa Suffrian

  Adults very common, feeding on the leaves of *Stigmaphylon lingulatum* on the plateau, March 7 and July 20, 1944.

Megistops lituratus Olivier

  Martorell collected specimens on *Clusia rosea*, April 2, 1940 (Acc. No. 311–40).

Chalepus sanguinicolis Linnaeus

  One specimen swept from weeds, Sardinera Beach, July 20, 1944.

Family ANTHRIBIDAE

Toxotropis sp.

  Det. L. L. Buchanan

  A single specimen swept from vegetation, Sardinera Beach, August 11–31, 1944.

Family CURCULIONIDAE

Cylas formicarius Fabricius

  One specimen at light, Sardinera Beach, April 5, 1944.

Artipus monae Wolcott

  Described from Mona by Wolcott 1941: 102–103 from 15 specimens taken on August 8, 1939 on casuarina foliage and eggplant leaves. The writer has numerous specimens swept from mixed vegetation at Sardinera and Uvero Beaches, March 4–6, April 4–7, July 21, and August 11–31, 1944. Martorell observed adults feeding on the leaves of *Amyris elemifera* on April 6, 1944 (Acc. No. 37–44). Specimens in the collection of the College of Agriculture, Mayaguez, Puerto Rico, collected on the island by the author in April, 1935 and determined as *Artipus* sp. by L. L. Buchanan, are included under this species.

Diaprepes abbreviatus Linnaeus

  Wolcott 1941: 103 reports adults feeding on the young leaves of *Terminalia Catappa* at Sardinera Beach, April 4, 1940.

Lachnopus kofresi Wolcott

  Wolcott 1941: 104 described this species from 22 specimens taken on cultivated eggplants at Rancho Grande, August 8, 1939. The writer has

numerous specimens swept from vegetation at various localities on April 4–7, June 29, July 19 and 21, and August 11–31, 1944.

Apodrosus argentatus Wolcott

Wolcott 1941: 104 reports specimens taken from shoots of *Colubrina colubrina* at Sardinera Beach, April 1, 1940.

Anthonomus sp.

Several specimens swept from vegetation at Uvero Beach, March 7, 1944.

Pseudomopsis sp.

Det. L. L. Buchanan
One specimen swept from vegetation, Sardinera Beach, June 29, 1944.

Family PLATYPOIDAE

Platypus rugulosus Chapuis

One specimen at light, Sardinera Beach, March 7, 1944.

Family SCOLYTIDAE

Xyleborus confusus Eichoff

Det. W. H. Anderson
Adults at light, Sardinera Beach, April 7, and August 11–31, 1944.

Order LEPIDOPTERA

Family EUCHROMIIDAE

Eunomia rubripunctata Butler

Wolcott 1941: 125 reports adults at light, Sardinera Beach, March 30, 1940.

Family ARCTIIDAE

Ammalo insulata Walker

Adults reported by Wolcott 1941: 125 at light at the Lighthouse, April 1, 1940.

Calidota strigosa Walker

Reported by Wolcott 1941: 125 as abundant at light, Sardinera Beach and the Lighthouse, March 30, 1940.   Adults collected in large numbers at light at Sardinera Beach, March 3–7 and April 4–7, 1944.

Family PERICOPIDAE

Composia sybaris Cramer

Wolcott 1941: 125 reports this species at light at Sardinera Beach, March

29–30, 1940. The writer secured numerous adults from the flowers of *Pisonia albida* at Uvero Beach, April 4–7, 1944.

Family NOCTUIDAE

Feltia subterranea Fabricius

Wolcott 1941: 126 reports the larvae of this species (as *F. annexa* Treitschke) on weeds at Sardinera, August 6, 1939. Numerous adults collected at light, Sardinera Beach, April 4–7 and August 11–31, 1944.

Catabena esula Druce

Reported by Wolcott 1941: 126 as common at light, Sardinera Beach and the Lighthouse, March 30 and April 1, 1940. The writer collected several adults at light, Camp Cofresí, April 4, 1944.

Micrathetis triplex Walker

Det. W. T. M. Forbes

A single adult taken at light, Sardinera Beach, April 4–7, 1944.

Eutelia piratica Schaus

Det. W. T. M. Forbes

One adult specimen collected at light, Sardinera Beach, April 4–7, 1944.

Mocis latipes Guenée

Det. W. T. M. Forbes

Adults collected at light, Sardinera Beach, August 11–31, 1944.

Mocis megas Guenée

Det. W. T. M. Forbes

Adults common at light, Sardinera Beach, August 11–31, 1944.

Plusia oo Fabricius

Det. W. T. M. Forbes

A single adult taken at light, Sardinera Beach, August 11–31, 1944.

Melipotis contorta Guenée

Det. W. T. M. Forbes

Several adults taken at light, Sardinera Beach, March 4, April 4–7, and June 29, 1944.

Melipotis famelica Guenée

Det. W. T. M. Forbes

Adults taken at light, Sardinera Beach, April 4–7 and August 11–31, 1944.

Melipotis januaris Guenée

Det. W. T. M. Forbes

Several adults collected at light, Sardinera Beach, April 4–7, 1944.

Melipotis fasciolaris Hübner

Det. W. T. M. Forbes

A single adult specimen taken at light, Sardinera Beach, April 4–7, 1944.

Hypenula complectalis Grote

Det. W. T. M. Forbes

Adults common at light, Sardinera Beach, April 4–7, June 29 and July 20, 1944.

Pseudohemiceras krugii Möschler

Wolcott 1941: 127 reports caterpillars of this species boring in the twigs of *Tabebuia lucida* and *Tabebuia heterophylla* on the plateau, April 1, 1940.

Glympis (Aluaca) eubolialis Walker

Det. W. T. M. Forbes

A single adult taken at light, Sardinera Beach, March 4, 1944.

Bendis gurda Guenée

Det. W. T. M. Forbes

Several specimens collected at light, Sardinera Beach, March 4, April 4–7, and June 29, 1944.

This species was previously recorded only from St. Thomas, Virgin Islands. According to Schaus 1940: 265 this species was unknown.

Azeta repugnalis Hübner

Det. W. T. M. Forbes

A single adult specimen collected at light, Sardinera Beach, April 4, 1944.

Epidromia pyraliformis

Det. W. T. M. Forbes

Adults taken at light, Sardinera Beach, June 21 and July 22, 1944.

Bleptina atymnusalis Walker

Det. W. T. M. Forbes

One specimen at light, Sardinera Beach, August 11–31, 1944.

Bleptina acastusalis Walker

Det. W. T. M. Forbes

Two specimens taken at light, Sardinera Beach, March 4, 1944.

Family NOTODONTIDAE

Nystalea ebalea Cramer

Det. W. T. M. Forbes

One specimen taken at light, Sardinera Beach, March 4, 1944.

Family SPHINGIDAE

Phlegethontius sextus jamaicensis Butler

Adults reported at light at the Lighthouse, April 1, 1940 by Wolcott 1941: 128.

Pseudosphinx tetrio Linnaeus

First reported from Mona by Leonard 1933: 135. Wolcott 1941: 129 reports one adult at light, the Lighthouse, and larvae on *Plumiera obtusa*, April 1, 1940. The writer has several adults taken at light at Sardinera Beach, March 4 and April 4–7, 1944. He also found practically all the *Plumiera* bushes on the plateau defoliated by the larvae.

Erinnyis ello Linnaeus

A single adult apecimen collected at light, Sardinera Beach, June 29, 1944.

Cautethia noctuiformis Walker

Wolcott 1941: 129 reports this species at light, Camp Cofresí, August 5, 1939.

Pachylia ficus Linnaeus

A single adult collected at light, Sardinera Beach, March 4, 1944.

Celerio lineata lineata Fabricius

Wolcott 1941: 129 reports specimens taken at light, Sardinera Beach, April 1, 1940.

Aëllopos tantalus Linnaeus var. **zonata** Drury

Martorell collected an adult on flowers of *Moringa moringa* at Sardinera Beach, April 1, 1940 (Acc. No. 315–40).

Family GEOMETRIDAE

Almodes terraria Guenée

Det. W. T. M. Forbes

Numerous specimens collected at light, Sardinera Beach, June 29 and July 20, 1944.

The description of the following new species was prepared by Dr. W. T. M. Forbes, Cornell University, who kindly gave his permission to include it with this report. The species should be credited to him.

Ptychopoda monata Forbes, n. sp.

General structures normal for the genus. Hind tibia much shorter than middle one, very thin and flimsy, but hollow and containing a large hair-pencil; femur linear; tarsus of five rounded segments, the first wider than tibia, then regularly decreasing; female hind tibia normal, with end spurs

only. Fore wing with accessory cell short and very slender, R_{2-4} and R_5 connate from its lower angle, R_1 also from its apex, but a little separated; R_4 apparently absent. Hind wing rather trapezoidal, sharply bent rather above M_3, the margin nearly erect above, but strongly oblique below, so that inner margin is only a little longer than part of outer margin below the bend. R and M_1 strongly stalked; male with a groove on under side along outer half of inner margin and around anal angle, filled with large spatulate scales attached close to inner margin.

Luteous. Face and palpi blackish, occiput with a slight transverse fuscous shade; legs shaded with fuscous, the fore legs mostly fuscous with contrasting luteous front tibia. Fore wing luteous, the outer third sometimes shaded with light fuscous or pale reddish brown, leaving a vague pale subterminal shade. Antemedial line heavy, black, excurved below costa, and toothed out on anal, incurved across submedian area and slanting in to inner margin; postmedial line heavy, strongly excurved opposite cell, a little incurved toward costa, and deeply incurved in a single sweep on lower half, slanting out again to inner margin. Discal dot a small oblique bar on lower part of discocellular. Medial area on lower half of wing often more or less shaded with black, sometimes almost solidly filled with black; the costal part sometimes shaded with red-brown about the discal bar. A series of small black terminal dots. Hind wing gray, with dark gray discal dot and a vague blackish median band running from it to middle of inner margin, sometimes reduced to a small triangular patch at inner margin; terminal dots as on fore wing. Abdomen shaded with red-brown above, with a few black scales. Expanse 11–12 mm.

Mona Island; a good series collected by J. A. Ramos in April 4–7, 1944; J. A. Ferrer, July 20, 1944; and L. F. Martorell in August, 1939. None of the specimens is in good condition, suggesting the species may be abnormally slow to die in the cyanide. Holotype, April 4–7, 1944 (Ramos) in the collection of the College of Agriculture, University of Puerto Rico, Mayaguez, Puerto Rico; paratypes in that collection and also the collection of Cornell University.

In the present state of confusion of the Sterrhinae it is not possible to be quite sure this species is not already described; but it is not represented in the National Museum, which is well supplied with West Indian material, nor in the Cornell University material from Puerto Rico and St. Croix. It should be distinguished from all the *Ptychopodas* known to me by the contrasting ordinary lines.

Racheospila sanctae-crucis Prout
    Det. W. T. M. Forbes
    Numerous adults taken at light, Sardinera Beach, June 29, 1944.

Racheospila cupedinaria Grote
Det. W. T. M. Forbes
A single specimen taken at light, Sardinera Beach, June 29, 1944.

Eucrotis sp.
Det. W. T. M. Forbes
Two specimens collected at light, Sardinera Beach, March 4, 1944.

Numia terebintharia Guenée
Det. W. T. M. Forbes
Several specimens taken at light, Sardinera Beach, April 4–7, July 20, and August 11–31, 1944.

Drepanodes infensata Guenée
Det. W. T. M. Forbes
Several specimens collected at light, Sardinera Beach, April 4–7 and July 20, 1944.

Family PYRALIDIDAE

Samea multuplicalis Guenée
Det. W. T. M. Forbes
Several specimens taken at light, Sardinera Beach, April 4–7, 1944.

Pilocrocis lauralis Walker
Wolcott 1941: 130 reports adults of this species at light, Camp Cofresí, August 7, 1939. The author has several adults also collected at light at the same locality, March 4, 1944.

Mesocondyla concordalis Hübner
Wolcott 1941: 130 records the larvae of this species on the leaves of *Tabebuia heterophylla* and *Tabebuia lucida* on the plateau, March 30, 1940. The writer has numerous adults of the pale variety (Det. W. T. M. Forbes) collected at light, Sardinera Beach, April 4–7 and August 11–31, 1944.

Dichogama amabilis Möschler
Adults reported at light, Camp Cofresí, August 7, 1939 and Sardinera Beach, April 1, 1940 by Wolcott 1941: 130. The writer collected several specimens at light at the same place on April 4–7, 1944.

Dichogama fernaldi Möschler
Det. W. T. M. Forbes
A single adult specimen taken at light, Sardinera Beach, June 29, 1944.

Dichogama redtenbacheri Lederer
Wolcott 1941: 131 reports a single adult collected at light, Sardinera Beach, March 30, 1940. The author collected numerous adults also at light at the same locality on April 4–7, 1944.

Lamprosema inabsconsalis Möschler
  Det. W. T. M. Forbes
  A single specimen at light, Sardinera Beach, April 4, 1944.

Margaronia costata Fabricius
  Wolcott 1941: 131 reports adults at light, Camp Cofresí, August 6–7, 1939, and at Sardinera Beach, April 1, 1940. The writer has adults collected also at light, Sardinera Beach, April 4–7 and June 29, 1944. He found the larvae on the leaves of *Rawolfia nitida* at Sardinera, April 5, 1944.

Hellula phidilealis Walker
  Det. W. T. M. Forbes
  Numerous specimens taken at light, Sardinera Beach, March 4, April 4–7, and July 20, 1944.

Crocidophora algarrobalis Schaus
  Det. W. T. M. Forbes
  A single adult specimen collected at light, Sardinera Beach, July 20, 1944.

Psara phaeopteralis Guenée
  Det. W. T. M. Forbes
  Two specimens at light, Sardinera Beach, June 29, 1944.

Loxostege similalis Guenée
  Det. W. T. M. Forbes
  Many specimens taken at light, Sardinera Beach, July 20, 1944.

Crambus santiagellus Schaus
  Det. W. T. M. Forbes
  One specimen at light, Sardinera Beach, April 4, 1944.

Crambus fissiradiellus Walker
  Det. W. T. M. Forbes
  Two adults collected at light, Sardinera Beach, April 4, 1944.

Jocara sp.
  Larvae attacking the leaves of *Conocarpus erecta*, south of Uvero Beach, April 5, 1944.

Scirpophaga longicornis Möschler
  Reported at light at Sardinera Beach, August 5, 1939 by Wolcott 1941: 132.

Diatraea saccharalis Fabricius
  Wolcott 1936: 475 reports the larvae on sugar cane without definite

locality or date.   Later (1941:132) he reports one adult at light at Sardinera Beach, April 1, 1940.

Elasmopalpus lignosellus Zeller
Det. W. T. M. Forbes
One adult specimen at light, Sardinera Beach, April 4, 1944.

Ephestiodes sp.
Det. W. T. M. Forbes
Several specimens taken at light, Sardinera Beach, July 20, 1944.

Family PTEROPHORIDAE

Trichoptilus defectalis Walker
Det. W. T. M. Forbes
One specimen taken at light, Sardinera Beach, June 29, 1944.

Family COSSIDAE

Psychonoctua personalis Grote
Wolcott 1941: 135 reports one adult at light, Camp Cofresí, August 6, 1939.   Martorell and the writer found larvae boring in the trunk of *Coccolobis uvifera* at Uvero Beach, April 6, 1944.

Family GELECHIIDAE

Aristotelia diolcella Forbes
Det. W. T. M. Forbes
Several specimens collected at light, Sardinera Beach, March 4, 1944.

Stegasta capitella Fabricius
Det. W. T. M. Forbes
Specimens collected at light, Sardinera Beach, March 4, 1944.

Pectinophora gossypiella Saunders
Wolcott 1941: 136 reports a heavy infestation by the larvae of this species on wild cotton at Rancho Grande and Uvero Beach, August 5, 1939.   During their visit to the island on April 4–7, 1944, Wolcott, Martorell, and the writer examined several plants of wild cotton at Uvero Beach and full-sized larvae were noted by them.   Although only a few bolls were found to be attacked, it was presumed that the infection was general and that every plant was infested.

Family ETHMIIDAE

Ethmia notatella Walker
Wolcott 1941: 136 reports this species abundant at light at Camp Cofresí, August 6, 1939 and at Sardinera Beach and the Lighthouse, April

1, 1940. The writer found the species very abundant at light at Sardinera Beach, April 4–7, 1944. He has also numerous adults collected at light at that locality on March 4, 1944.

Family PSYCHIDAE

Oiketicus kirbyi Guilding

Wolcott 1941: 137 reports this bagworm on casuarinas at Sardinera Beach, August 5, 1939. The writer also noted the insect on the same host at the same locality and on *Pisonia albida* at Uvero Beach, April 7, 1944.

Family TINEIDAE

Tineola uterella Walsingham

Wolcott 1941: 137 reports the larvae on the walls of houses at Sardinera Beach, April 1, 1940. The writer also observed them abundant in houses at the same locality on April 4–7, 1944.

Family NEPTICULIDAE

Nepticula gossypii Forbes

Collected by Wolcott on wild cotton at Uvero Beach on September, 1944 (personal correspondence).

Family DANAIDAE

Danaus plexippus plexippus Linnaeus

One male collected on the plateau above Sardinera Beach, July 20, 1944.

Family NYMPHALIDAE

Heliconius charithonius charithonius Linnaeus

Wolcott 1941: 122 reports specimens flying in shaded places near the cliff, Sardinera Beach, August 7, 1939. The writer has several specimens collected at Sardinera Beach, March 5, and July 19–22, 1944, when the species was observed to be rather common.

Dione vanillae insularis Maynard

Recorded by Wolcott 1941: 122 at Sardinera Beach, April 1, 1940. Common at Sardinera and Uvero Beaches and on the plateau on April 4–7, June 29 and July 17–22, 1944, when numerous specimens were collected. Larvae taken at Uvero on *Corchorus hirsutus*, July 19, were bred to adults.

Junonia evarete zonalis C. and R. Felder

One specimen taken on the plateau, July 20, 1944.

Junonia evarete genoveva Cramer

Several specimens taken on the plateau, July 20, 1944.

Hypolimnas misippus Linnaeus

Wolcott (in correspondence) collected this species on September 1944.

Eunica monima Cramer

Wolcott 1941: 123 reports this species from Sardinera Beach, March 29, 1940.

Hamadryas ferox diasia Fruhstorfer

Det. W. P. Comstock

Four specimens collected at Sardinera Beach, August 11–31, 1944.

Family LYCAENIDAE

Hemiargus ammon noëli Comstock & Huntington

Det. W. P. Comstock

Comstock and Huntington 1941: 100 recorded a male of this Hispaniolan species captured on Mona Island, February 21–26, 1914 by F. E. Lutz. The writer has numerous specimens taken at Uvero Beach and on the plateau on the following dates: April 4–7 and July 29, 1944. Specimens taken at Sardinera Beach on April 1, 1940 and listed by Wolcott 1941: 123 as *Hemiargus* sp. near *zacheina* B. and D. undoubtedly belong to this species.

Family PIERIDAE

Phoebis (Phoebis) sennae sennae Linnaeus

Few adults were seen flying near the cliff at Sardinera Beach, April 4–7, 1944.

Eurema (Eurema) palmira palmira Poey

Det. W. P. Comstock

One specimen on the plateau, July 20, 1944.

Eurema (Pyrisitia) lisa euterpe Ménétriés

Det. W. P. Comstock

Two males, one white and one yellow female on the plateau, July 20, 1944.

Appias (Glutophrissa) drusilla boydi Comstock

Det. W. P. Comstock

Comstock 1944: 527 mentioned Mona Island in the distribution of this species. The writer has the following specimens in his collection, taken near the cliff at Sardinera Beach: 2 males, March 5, and another on June 29, and 1 female, April 4–7, 1944. Comstock's determination of the female specimen is accompanied by the following remark: "This is a very lightly marked female such as occurring in Hispaniola".

Ascia monuste eubotea Latreille

Det. W. P. Comstock

Two males and one female at Sardinera Beach, June 29, 1944. Comstock accompanies his determination of the female specimen with the remark: "This is a dark female like many from Hispaniola".

In following Comstock's (1944: 529) interpretation of the distribution of the forms of this species, the writer includes Wolcott's record of *monuste* from Mona Island ("attacking onions when normal host was weeded out," 1943: 123) under this form.

Family HESPERIIDAE

Urbanus proteus Linnaeus

Wolcott 1941: 124 recorded adults abundant on flowers of *Moringa moringa* and *Pisonia albida* at Sardinera Beach, April 1, 1940.

Urbanus dorantes cramptoni Comstock

Det. W. P. Comstock.

Comstock 1944: 546–547 designed 3 specimens from Mona Island as paratypes for his description of **cramptoni**: 2 males and 1 female, February 21–26, 1914. The writer has in his collection 2 specimens collected at Sardinera Beach, March 5 and April 7, 1944.

Pyrgus syrichtus Fabricius

Recorded by Wolcott 1941: 124 at Sardinera on August 8, 1939 and March 30, 1940. Four specimens in the author's collection (Det. W. P. Comstock) were collected on the plateau, August 11–31, 1944.

Ephyriades arcas Drury

Det. W. P. Comstock

One specimen collected on the plateau, July 21, 1944.

Wallengrenia otho mutchleri Watson

Wolcott 1942: 124 recorded specimens taken at Sardinera Beach, August 8, 1939 and March 30, 1940.

Lerodea tripuncta Herrich-Schäffer

Wolcott 1941: 124 reports this species at Sardinera Beach, August 7 1939. The writer has several specimens (Det. W. P. Comstock) taken at Sardinera Beach, August 11–31, 1944.

Panoquina nyctelia Latreille

On weeds at Sardinera Beach, August 6, 1939 (Wolcott 1941: 124).

Order DIPTERA
Family CULICIDAE

Aëdes aegypti Linnaeus

Curran 1928: 10 reports four males collected on Mona Island by F. E.

Lutz, February 21–26, 1914. The writer found this mosquito common and troublesome at Sardinera Beach, April 4–7, 1944.

Culex fatigans Wiedemann

This species was found by the writer extremely abundant and troublesome at Sardinera Beach, April 4–7, 1944.

Family CECIDOMYIDAE

Cecidomyia coccolobae Cook

Wolcott 1941: 112 reports this species as making small cone-shaped galls on the leaves of *Coccolobis uvifera*, August 9, 1939.

Family STRATIOMYIDAE

Neorondania chalybea Wiedemann

Wolcott 1941: 112 records this species as abundant in houses and latrines at Camp Cofresí, August 8, 1939 and at the Lighthouse, April 1, 1940.

Nemoteles monensis Curran

Described by Curran 1928: 16 from a single female taken on Mona Island by F. E. Lutz on February 21–26, 1914.

Family TABANIDAE

Tabanus caribaeorum Bequaert

This species was described from Grand Cayman and Mona Island by Bequaert 1940: 323–326. The two paratypes from Mona, a male and a female, were collected in 1940 by L. F. Martorell.

Tabanus stigma Fabricius

Wolcott 1941: 113 reports this species collected at Camp Cofresí, August 6, 1939.

Family BOMBYLIIDAE

Hyperalonia cerberus Fabricius

Curran 1928: 19 reports specimens taken by F. E. Lutz on February 21–26, 1914 and Wolcott 1941: 113 reports specimens collected at Playa de Pájaros, Uvero Beach, and Camp Cofresí, August 7, 1939. The writer has several specimens taken at Sardinera Beach and on the plateau, August 11–31, 1944.

Spongostylum sp. near pluto Wiedemann

Wolcott 1941: 113 reports specimens taken at Playa de Pájaros, August 8, 1939.

Heterostylum ferrugineus Fabricius

Wolcott 1941: 114 reports one specimen taken in a cave, August 8, 1939. The writer collected one specimen on weeds at Sardinera Beach, April 6, 1944.

Exoprosopa sp. near **dodrans** Osten Sacken

Wolcott 1941: 114 reports specimens taken on weeds, Playa de Pájaros, August 8, 1939.

Villa lateralis Say

Specimens taken by F. E. Lutz on February 21–26, 1914 are reported by Curran 1928: 20.

Villa gorgon Fabricius

Curran 1928: 21 reports this species from specimens collected on February 21–26, 1914 by F. E. Lutz. Wolcott 1941: 114 reports additional specimens collected on August 8, 1939 and April 1, 1940. The writer found the species rather common at Sardinera and Uvero Beaches and on the plateau on April 4–7, 1944. He has other specimens collected on August 11–31, 1944.

Family ASILIDAE

Ommatius marginellus Fabricius

Recorded by Wolcott 1941: 114 on weeds at Rancho Grande, August 7, 1939.

Leptogaster cubensis Bigot

Known from Mona only by two specimens collected on February 21-26, 1914 by F. E. Lutz and reported by Curran 1928: 22.

Plesioma sp. near **indecora** Leow

Reported by Wolcott 1941: 114 on weeds at Camp Cofresí, August 6, 1939.

Family THEREVIDAE

Psilocephala monensis Curran

Described by Curran 1926:2 from a single specimen collected by F. E. Lutz on February 21–26, 1914.

Psilocephala vexans Curran

Originally described by Curran 1926: 2 from a series of specimens from Puerto Rico and other West Indian Islands. Two of the paratypes were taken at Mona on February 21–26, 1914.

Family DOLICHOPODIDAE

Thrypticus violaceus Van Duzee

Van Duzee (in Curran 1928: 30) reports specimens of this species from Mona taken on February 21–26, 1914.

Sciapus albiciliatus Van Duzee

Originally described by Van Duzee 1927: 9–10 from specimens from

Puerto Rico, Virgin Islands and one from Mona collected by F. E. Lutz, February 21–26, 1914.

Psilopus sp. near insularis Aldrich

Det. C. T. Greene

Martorell collected specimens on weeds at Sardinera Beach, April 1, 1940 (Acc. No. 292–40).

Family SYRPHIDAE

Baccha conformis Leow

Wolcott 1941: 115 reports specimens taken at Sardinera Beach, August 7, 1939 and on the plateau, April 1, 1940.

Baccha cylindrica Fabricius

Curran 1928: 26 lists specimens collected on the island by F. E. Lutz on February 21–26, 1914. The writer has specimens taken at Sardinera Beach on August 11–31, 1944.

Baccha fasciata Roeder

Wolcott 1941: 115 reports this species taken on weeds at Sardinera Beach, April 1, 1940.

Allograpta fuscisquama Curran

Curran 1927: 5 included a male taken on Mona Island by F. E. Lutz on February 21–26, 1914, as a paratype in his description of this species.

Allograpta limbata Fabricius

Wolcott 1941: 115 reports this species as collected at Sardinera Beach, March 30, 1940.

Volucella horvathi Szilady

Wolcott 1941: 115 records this species flying in the shade of trees at Sardinera Beach and Playa de Pájaros, August 8, 1939.

Family PHORIDAE

Megaselida scalaris Leow

Curran 1928: 43 reports numerous specimens from Mona Island collected on February 21–26, 1914.

Syneura cocciphila Coquillett

Wolcott obtained several adults from *Icerya purchasi*, April 6, 1944 (Acc. No. 62–44).

Family CHLOROPIDAE

Prohippelates pallidus Leow

This Cuban species is known from Mona by a single specimen collected

on the island on February 21–26, 1914 by F. E. Lutz and reported by Curran 1928: 45.

Hippelates dorsatus Williston

Curran 1928: 46 reports three specimens collected on Mona, February 21–26, 1914.

Hippelates tener Coquillett

One specimen taken on the island on February 21–26, 1914 by F. E. Lutz is reported by Curran 1928:47.

Hippelates convexus Loew

Curran 1928: 49 reports specimens collected on February 21–26, 1914.

Hippelates flavipes Loew

Curran reports specimens collected by F. E. Lutz on February 21–26, 1914.

Hippelates lutzi Curran

Described from Mona Island by Curran 1926: 5 from specimens taken there by F. E. Lutz on February 21–26, 1914.

Hippelates bicolor Coquillett

Curran 1928: 49 reports specimens collected on the island on February 21–26, 1914 by F. E. Lutz.

Hippelates collusor Curran

A female collected on Mona Island by F. E. Lutz on February 21–26, 1914 was designed by Curran 1926: 4 as a paratype in his description of this species.

Hippelates pusio Loew

Curran 1928: 49 reports specimens collected on the island on February 21–26, 1914.

Hippelates apicata Malloch

Curran 1928: 50 lists specimens from Mona Island collected on February 21–26, 1914.

Botanobia limitata Becker

Specimens collected in Mona Island on February 21–26, 1914 are reported by Curran 1928: 53.

Botanobia sicatrix Curran

Described from Mona Island by Curran 1926: 8 from 17 specimens collected on February 21–26, 1914.

Botanobia mona Curran

Curran 1926: 9 described this species from Mona Island from specimens collected there on February 21–26, 1914.

Botanobia mars Curran

Curran 1926: 10 designed two females collected on Mona Island, February 21–26, 1914, as paratypes in his description of this species.

Botanobia tripunctata Curran

The original description of this species by Curran 1926: 10 is based on three specimens collected on Mona Island on February 21–26, 1914.

Botanobia varipalpus Curran

Another species described by Curran 1926: 12 from specimens collected on Mona Island on February 21–26, 1914.

Family EPHYDRIDAE

Ceropsilopa coquilletti Cresson

A single specimen taken on the island on February 21–26, 1914 is listed by Curran 1928: 61.

Plagiops aciculata Loew

Listed by Curran 1928: 62 as collected on the island on February 21–26, 1914.

Discocerina obscurella Fallen

Curran 1928: 63 reports a single specimen from Mona Island, collected on February 21–26, 1914.

Family AGROMYZIDAE

Agromyza aeneiventris Fallen?

Wolcott 1941: 121 reports this species on tender leaves of *Coccolobis laurifolia* (Acc. No. 43–40).

Cryptochaetum iceryae Williston

Adults from a shipment of parasitized cottony cushion scales from California released in the island in 1940 (Wolcott 1941: 121).

Family OCHTHIPHILIDAE

Acrometopia maculata Coquillett

Curran 1928: 66 reports seven specimens taken on the island on February 21–26, 1914.

Family MICHILIDAE

Michiella lacteipennis Loew

Curran 1928: 67 lists one specimen taken in the island on February 21–26, 1914.

Pholeomyia indecora Loew

A single specimen collected in the island on February 21–26, 1914 is reported by Curran 1928: 68.

Family TRYPANEIDAE

Tetraeuaresta obscuriventris Loew

Wolcott 1941: 120 reports one specimen collected on weeds at Sardinera Beach, April 1, 1940.

Family SEPSIDAE

Sepsis pusio Schiner

Curran 1928: 76 reports specimens collected in the island on February 21–26, 1914.

Family OTITIDAE

Euxesta stigmatias Loew

Specimens collected in the island on February 21–26, 1914 are reported by Curran 1928: 78.

Euxesta abdominalis Loew

Curran 1928: 79 reports specimens collected in Mona on February 21–26, 1914.

Euxesta annonae Fabricius

Curran 1928: 79 reports specimens taken on the island on February 21–26, 1914.

Notogramma stigma Fabricius

Specimens collected in the island on February 21–26, 1914 are reported by Curran 1928: 79.

Family SAPROMYZIDAE

Carpolonchaea pendula Bezzi

Wolcott 1941: 118 reports this species taken on weeds at Camp Cofresí, August 5, 1939 and at Sardinera Beach, April 1, 1940.

Camptoprosopella diversa Curran

Curran 1928: 82 lists one specimen taken on Mona, February 21–26, 1914.

Neogriphoneura sordida Wiedemann

Two specimens collected on the island, February 21–26, 1914, are listed by Curran 1928: 82.

Minettia slossonae Coquillett

Curran 1928: 84 reports specimens collected on February 21–26, 1914.

Minettia mona Curran

Described by Curran 1926: 13–14 from 6 specimens from Mona Island, February 21–26, 1914, and 3 from Puerto Rico.

Family MUSCIDAE

Musca domestica Linnaeus

Wolcott 1941: 117 gives several records for the housefly from Mona on August 6, 1939; March 29 and April 5, 1940. The writer found the species very abundant in houses in April 4–7, 1944.

Family CALLIPHORIDAE

Cochliomyia macellaria Fabricius

First reported from the island by Curran 1928: 92, who lists specimens collected on February 21–26, 1914. Wolcott 1941: 117 reports that this fly is so abundant and troublesome on the island as to prevent drying of fish on the beach.

Cochliomyia laniaria Wiedemann

Curran 1928: 92 reports specimens taken on February 21–26, 1914. Martorell collected the species on flowers of *Colubrina colubrina* on the plateau, April 1, 1940 (Acc. No. 284–40).

Lucilia eximia Macquart

Martorell secured one specimen, determined as this species by D. G. Hall, at Sardinera Beach, March 30, 1940 (Acc. No. 277–40).

Family SARCOPHAGIDAE

Sarcophaga bakeri Aldrich

Curran 1928: 99 reports specimens collected on February 21–26, 1914.

Sarcophaga currani Hall

Wolcott 1941: 117 reports specimens taken at the Viejo Lirio cave, Playa de Pájaros, August 9, 1939.

Sarcophaga rapax Walker

Det. M. T. James

Specimens obtained by Wolcott from a dead adult of *Strataegus barbigerus* collected between Sardinera and Uvero Beaches, October 2, 1944 (Acc. No. 196–44).

Helicobia globulus Aldrich

Curran 1928: 100 lists specimens taken on February 21–26, 1914.

Helicobia helicis Townsend

Specimens taken in the island, February 21–26, 1914, are listed by Curran 1928: 101.

Sarcophagula occidusa Fabricius

Curran 1928: 101 reports specimens from Mona, February 21–26, 1914.

Harpagopyga diversipes Coquillett

Curran 1928: 102 lists one specimen from the island, February 21–26, 1914.

Sarothromyia femoralis Schiner

Wolcott 1941: 117 reports this species at light (Acc. No. 374–39).

Senotainia rubriventris Macquart

Curran 1928: 104 reports a single specimen from the island, February 21–26, 1914.

Family HIPPOBOSCIDAE

Olfersia spinifera Leach

Recorded by Wolcott 1941: 121 from man-o'-war bird, *Fregata magnificens rothschildi* Mathews, Sardinera Beach, August 6, 1939.

Order SIPHONAPTERA

Family HECTOPSYLLIDAE

Tunga penetrans Linnaeus

On man, Sardinera Beach, August 6, 1939 (Wolcott 1941: 122).

Family PULICIDAE

Ctenocephalides canis Curtis

Wolcott 1941: 122 reports this flea on dogs, Sardinera Beach, August 5, 1939.

Order HYMENOPTERA

Family ICHNEUMONIDAE

Tromatobia lateralis Cresson

Det. H. K. Townes

One specimen at Uvero Beach, August 11–31, 1944.

Family BRACONIDAE

Apanteles sp.

Det. C. F. W. Muesebeck

One specimen, Sardinera Beach, April 5, 1944.

Iphiaulax sp.

Martorell collected three specimens at Camp Cofresí, August 6, 1939 (Acc. No. 188–39).   The writer has one specimen, determined by C. F. W. Muesebeck, taken on the plateau, July 21, 1944.

Trigonophasmus sp. nov.

Wolcott 1941: 140 reports one specimen collected on the plateau, April 1, 1940.

Family CHALCIDIDAE

Brachymeria incerta Cresson

Wolcott 1941: 147 records one specimen taken on the plateau, April 1, 1940.

Ceratomiscra debilis Cresson

Det. A. B. Gahan

One specimen on the plateau, August 11–31, 1944.

Spilochalcis flavopicta Cresson

Wolcott 1941: 147 reports one specimen collected at Camp Cofresí, August 7, 1939.   The writer collected one specimen (Det. A. B. Gahan) at Sardinera Beach, April 5, 1944.

Spilochalcis homaledrae Wolcott

Martorell collected one specimen on the flowers of *Pisonia albida* on the plateau, April 2, 1940 (Acc. No. 308–40).

Family CALLIMOMIDAE

Megastigmus sp. nov.

Det. A. B. Gahan

One specimen on the plateau above Uvero Beach, August 11–31, 1944.

Family PTEROMALIDAE

Pachyneuron allograptae Ashmead

Wolcott 1941: 144 reports this species from Rancho Grande on syrphid fly puparia, March 31, and resting on watermelons, March 30, 1940.

Family EUPELMIDAE

Eupelmus sp. a.

Det. A. B. Gahan

One specimen, April 4; and 3 specimens, August 11–31, 1944.

Eupelmus sp. b.

Det. A. B. Gahan

One specimen, April 7, 1944.

Anastatus sp.

Det. A. B. Gahan

Reared by Wolcott from egg masses of *Callimantis antillarum* taken at Camp Cofresí, October 3, 1944 (Acc. No. 197–44).

Family EULOPHIDAE

Tetrastichus sp.

Det. A. B. Gahan

One specimen, Sardinera Beach, August 11–31, 1944.

Family SCELIONIDAE

Hoploteleia sp., "apparently new"

Det. C. F. W. Muesebeck

One specimen, Sardinera Beach, June 29, 1944.

Telenomus sp.

Det. A. B. Gahan

Specimens collected at Sardinera Beach, August 11–31, 1944.

Family DRYINIDAE

Gonatopus sp.

Det. C. F. W. Muesebeck

One specimen swept from weeds, Sardinera Beach, April 4, 1944.

Family FORMICIDAE

Dr. M. R. Smith, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, kindly determined all the species of ants from Mona Island in the author's collection.

Platythyrea punctata F. Smith

A single worker collected in the shade at the base of the cliff, Sardinera Beach, August 11–31, 1944.

Ponera opaciceps Mayr

Collected at Sardinera Beach, August 11–31, 1944.

Odontomachus haematodes insularis Guerin

Wolcott 1941: 148 reports this species nesting in a rotten stump at Sardinera Beach, August 7, 1939 and at light on the same locality, April 1, 1940. The writer found a nest in a rotten wild papaya trunk near the cliff at Sardinera Beach, April 4, and has many specimens swept from weeds at the same place, April 2 and August 11–31, 1944.

Monomorium floricola Jerdon

Wolcott 1941: 149 reports this species nesting in a stump at Camp

Cofresí, August 5, 1939. The writer has numerous specimens taken at Sardinera Beach, March 6 and August 11–31, 1944.

Monomorium pharaonis Linnaeus
Many workers swept from vegetation, Uvero Beach, August 11–31, 1944.

Cardiocondyla emeryi Forel
Specimens collected at Sardinera Beach, August 11–31, 1944.

Cardiocondyla venustula Wheeler
Smith 1944: 38 reports specimens taken on Mona Island on February 21–26, 1914.

Solenopsis geminata Fabricius
Wolcott 1941 : 149 reports this ant as abundant all over the island, August 5–7, 1939; and attending *Icerya purchasi* on casuarinas at Sardinera Beach, March 29, 1940. The writer observed the species attending *Coccus viridis* on *Rawolfia nitida* at Sardinera Beach, April 5, 1944.

Pheidole moerens Wheeler
Numerous specimens collected under debris in a shaded place near the base of the cliff, Sardinera Beach, August 11–31, 1944.

Macromischa albispina subsp. albipes Mann
Described by Mann 1920: 424 from Mona Island as a variety of *M. albispina* Wheeler. It was later raised to subspecific rank by Wheeler 1931 : 1–34. Smith 1937: 851 gives notes on this ant but does not give any records of new captures. The writer has specimens taken on the ground under the shade at the base of the cliff, Sardinera Beach, August 11–31, 1944.

Tetramorium guineense Fabricius
Many specimens swept from weeds, Sardinera and Uvero Beaches, April 5, 1944.

Wasmannia auropunctata Roger
Wolcott 1941: 149 records specimens on the ground, Camp Cofresí, August 5, 1939. The writer noted the species abundant on the young shoots of *Terminalia Catappa* at Sardinera Beach, April 5, 1944.

Dorymyrmex pyramicus var. niger Pergande
Wolcott 1941: 150 reports this species nesting in a stump at Camp Cofresí, August 5, 1939; and attending *Icerya purchasi* on casuarinas at Sardinera Beach, March 29, 1940. The writer found it common at Sardinera and Uvero Beaches, April 4–7, 1944. He has also numerous specimens swept from vegetation at the same localities, March 4, July 19, and August 11–31, 1944.

Trachymyrmex jamaicensis André
Nesting in the open ground at Los Cerezos on the plateau, July 21, and in shaded places near the cliff, Sardinera Beach, August 11–31, 1944.

Tapinoma melanocephalum Fabricius
Nesting in rotten stump of *Coccolobis laurifolia*, Sardinera Beach, April 5, 1944.

Prenolepis longicornis Latreille
Wolcott 1941: 150 records this species as abundant at Camp Cofresí, August 5–6, 1939.

Camponotus sp.
Nesting in a rotten stump of *Conocarpus erecta*, Uvero Beach, April 7, 1944.

Myrmelachista ramulorum subsp. **fortior** Wheeler
Wheeler 1934: 189–190 described the subspecies of this ant from 5 workers collected on Mona Island by F. E. Lutz on February 21–26, 1914, and 2 workers collected by him in Puerto Rico. Smith 1937: 873 discusses the species without giving any new records for Mona Island. The writer has numerous workers taken at Sardinera Beach, August 11–31, 1944.

Family BEMBECIDAE

Bicyrtes spinosa Fabricius
Wolcott 1941: 150 records a single specimen taken on weeds at Sardinera Beach, August 6, 1939. The writer has two specimens, determined by H. K. Townes, collected at Sardinera Beach, March 6 and July 19, 1944.

Stictia signata Linnaeus
Common all over the island (Wolcott 1941: 151). The writer has many specimens collected at Sardinera Beach, March 6, April 7 (attracted by dead bird), July 20, and August 11–31, 1944.

Family SPHECIDAE

Tachytes insularis Cresson
Wolcott 1941: 152 records specimens at the airfield, August 9, 1939 and also at Sardinera and on the plateau, April 1, 1940.

Tachytes sp.
Det. H. K. Townes
Four specimens taken at Uvero Beach and on the plateau, August 11–31, 1944.

Motes sp. a.

Det. H. K. Townes

A single specimen taken on the plateau above Uvero Beach, August 11–31, 1944. Townes' determination of this species is accompanied by the remark: "*Motes* sp. a. is like specimens in the National Museum determined as *Motes vinulentus* Cresson".

Motes sp. b.

Det. H. K. Townes

One specimen from the plateau, August 11–31, 1944. Townes remarks that: "This species is like specimens in the National Museum determined as *Motes trifasciatus* Smith".

Chlorion (Ammobia) singularis F. Smith

Wolcott 1941: 152 reports one specimen taken at Sardinera Beach, March 30, 1940.

Chlorion thomae Fabricius

Wolcott 1941: 152 reports this species at the airfield, August 8, 1939; and also abundant at the plateau, March 30, 1940. The writer has numerous specimens from Uvero Beach and the plateau, April 7, June 29, July 21, and August 11–31, 1944.

Trypoxylon sp.

Det. H. K. Townes

Numerous specimens taken on the plateau, August 11–31, 1944.

Family CRABRONIDAE

Crabro croesus Lepeletier

Listed by Wolcott 1936: 556 from Mona (Acc. No. 1308–13).

Family SCOLIIDAE

Elis haemorrhoidalis Fabricius

Wolcott 1941: 153 reports specimens taken on flowers of *Colubrina colubrina* and *Pisonia albida* at Sardinera Beach and on the plateau, March 30, 1940. The writer collected one specimen while sweeping on weeds at Uvero Beach, April 7, 1944.

Campsomeris atrata Fabricius

Recorded by Wolcott 1941: 154 at Sardinera Beach, August 7, 1939; and on flowers of *Moringa moringa, Pisonia albida*, and *Colubrina colubrina*, Sardinera Beach, March 30, 1940. The writer found the species abundant at Uvero Beach, April 5, 1944.

Campsomeris dorsata Fabricius

Wolcott 1941: 154 reports specimens on the ground at Sardinera Beach, August 6, 1939 and on flowers of *Moringa moringa,* March 30, 1940.

Family EUMENIDAE

Zethus rufinodus Latreille

Common on flowers of *Lantana* at Sardinera and Playa de Pájaros, August 6–8, 1939; and on the tender foliage of *Coccolobis laurifolia,* March 30, 1940 (Wolcott 1941: 156). The writer has specimens taken at Uvero and Sardinera Beaches, April 4 and July 20–21, 1944.

Family PSAMMOCHARIDAE

Cryptocheilus flammipennis Smith

Several specimens flying near the ground among weeds, Sardinera and Uvero Beaches, April 5–6, 1944.

Family VESPIDAE

Polistes crinitus Felton

Wolcott 1941: 155 records this wasp as abundant, nests on trees and other plants all over the island, August 5, 1939 and April 2, 1940. The writer found it rather scarce in April 4–7, 1944. He has one specimen dated August 11–31, 1944.

Polistes major Palisot de Beauvois

Nesting on sea-grape and casuarina trees, Playa de Pájaros and Sardinera Beach, August 8, 1939 (Wolcott 1941: 155). The writer noted a large nest on a *Lantana* bush at Sardinera Beach, April 6, 1944.

Mischocyttarus cubensis Saussure

Wolcott 1941: 156 reports specimens collected on weeds near Camp Cofresí, August 8, 1939. The author found a nest under a leaf of a tree at Sardinera Beach, April 4, 1944.

Pachodyneurus tibialis Saussure

Adults frequenting flowers of *Lantana* at Sardinera Beach and Playa de Pájaros, August 6, 1939; and of *Colubrina colubrina* at Sardinera Beach, March 30, 1940 (Wolcott 1941: 156). The writer has specimens, determined by H. K. Townes, from Sardinera Beach, July 21 and August 11–31, 1944.

Rygchium sp.

Det. H. K. Townes.

One male specimen on the plateau, July 21, 1944.

Family HALICTIDAE

Agapostemon portoricensis Cockerell

Wolcott 1941: 156 lists specimens frequenting flowers of *Lantana* at Playa de Pájaros and Sardinera Beach, August 6–7, 1939.

Halictus sp.

Martorell collected specimens by sweeping on weeds at Sardinera Beach, April 1, 1940 (Acc. No. 291–40). The writer collected several specimens also from weeds at the same locality, April 5, 1944.

Family ANTHOPHORIDAE

Centris haemorrhoidalis Fabricius

Wolcott 1941: 157 reports specimens on the flowers of *Moringa moringa* and *Pisonia albida* at Sardinera Beach and on the plateau, March 31, 1940.

Centris lanipes Linnaeus

On weeds at Sardinera Beach, August 7, 1939 and on flowers of *Moringa moringa*, *Colubrina colubrina* and *Pisonia albida*, Sardinera Beach and the plateau, March 30, 1940 (Wolcott 1941: 157).

Centris versicolor Fabricius

Wolcott 1941: 157 reports adults abundant on the flowers of *Lantana* at Playa de Pájaros, August 8, 1939 and of *Moringa moringa*, Sardinera Beach and the plateau, April 3, 1940.

Anthophora krugii Cresson

Adults reported by Wolcott 1941: 157 in walls of cave at Playa de Pájaros, August 8, 1939 and frequenting the flowers of *Moringa moringa* and *Colubrina colubrina*, Sardinera Beach, March 30, 1940.

Family MEGACHILIDAE

Megachile n. sp.

Det. T. B. Mitchell

Martorell collected two specimens of this new *Megachile* on the flowers of *Moringa moringa* and *Pisonia albida* at Playa de Pájaros, August 8, 1939. These specimens were reported as *Megachile vitrasi* Pérez by Wolcott 1941: 157. The writer has a single specimen taken at Sardinera Beach, August 11–31, 1944. All this material was examined by Dr. T. B. Mitchell, who found it to represent an undescribed species. A specimen taken by Wolcott at Guánica, Puerto Rico, August 24, 1939 (Acc. No. 225–39) and kindly loaned by him, constitutes a fourth specimen of this new *Megachile*.

Family XYLOCOPIDAE

Xylocopa brasilianorum Linnaeus

Abundant all over the island, August 7, 1939 (Wolcott 1941: 158).
The writer has specimens taken at Sardinera, March 3, 1944. He found
a nest with several adult males and females in an old branch of *Ficus
Stahlii* at Sardinera Beach, April 4, 1944.

DISCUSSION AND ANALYSIS OF THE INSECT FAUNA

In the present work, a total of 526 species of insects is recorded from
Mona Island. Of this number, 24 species, or 4.56 per cent, are endemic
to the island; 27 species, or 5.11 per cent, are also known only from the
Puerto Rican mainland; 53 species, or 10.07 per cent, although known from
other West Indian islands or other regions, are not known from Puerto
Rico itself; and 422 species, or 80.22 per cent, are widely ranging forms,
occurring in some or in all of the West Indies, or in neighboring regions
(Table I).

TABLE I

Order	No. of Families	Endemic species	In common with P. R.	Not known from P. R.	Of wide distribution	Total
1. *Thysanura*	1	0	0	0	1	1
2. *Collembola*..........	1	0	0	1	0	1
3. *Orthoptera*..........	8	3	1	0	24	28
4. *Dermaptera*..........	1	0	0	0	1	1
5. *Isoptera*............	1	1	1	1	1	4
6. *Neuroptera*..........	3	0	1	3	4	8
7. *Odonata*............	2	0	0	0	5	5
8. *Mallophaga*..........	1	0	0	0	1	1
9. *Thysanoptera*........	1	0	0	0	1	1
10. *Homoptera*..........	16	3	2	2	56	63
11. *Hemiptera*..........	16	3	1	4	49	57
12. *Coleoptera*..........	36	5	7	16	95	123
13. *Lepidoptera*.........	20	1	5	9	73	88
14. *Diptera*............	22	6	6	7	59	78
15. *Siphonaptera*........	2	0	0	0	2	2
16. *Hymenoptera*........	21	2	3	10	50	65
Totals................	152	24	27	53	422	526
Percentage............		4.56	5.11	10.07	80.22	

The Coleoptera is represented by 123 species of which 5 are endemic,
7 occur also only in Puerto Rico itself, and 16 are not known from the latter
island, although they occur also in other of the West Indies.

The Diptera has 78 representatives in this report. Of these, 6 species are endemic to Mona Island, 6 occur also only in Puerto Rico, and 7 range throughout other West Indian islands but not in Puerto Rico itself.

The Hymenoptera is represented by 65 species of which 2 are endemic, 3 are shared with Puerto Rico only and 10 occur in other of the West Indies but not Puerto Rico itself.

In the Homoptera, 63 species are recorded from the island of which 3 are endemic, 2 occur also in Puerto Rico only and 2 are not known from Puerto Rico itself although they occur in other localities. The family Kinnaridae, until recently not known from Puerto Rico itself, is represented in Mona by a new genus and species.

The Hemiptera is represented by 57 species of which 3 are endemic, one is shared with Puerto Rico only, and 4 do not occur in the latter island itself.

A total of 88 species is recorded in the Lepidoptera. Of these, one new species is described from the island, 5 are shared with Puerto Rico only and 9 with other regions but not Puerto Rico itself.

The Orthoptera is represented by 28 species of which 3 are endemic forms, one is shared with Puerto Rico only, and the rest are widely distributed in the West Indies.

Of the remaining orders of insects represented, the Isoptera is the only one having an endemic species from the island.

The paucity of the insect fauna of Mona Island, as shown by the above analysis, is probably due not only to the small area of the island but also to the extremely arid condition and scant vegetation of the region. Its most interesting feature is undoubtedly the fact that the number of species in common with other regions but not known from Puerto Rico itself (53 species, or 10.07 per cent) is nearly two times greater than the number of species in common with that island (27 species, or 5.11 per cent). This could be interpreted in the sense that the island's insect fauna has less affinities with that of Puerto Rico itself than with that of the other Greater Antilles. Unfortunately, the lack of a better knowledge of the insect faunas of these islands, especially of Hispaniola, does not permit a more definite statement in this respect.

SUMMARY

A total of 526 species of insects, representing 16 orders and 152 families, is recorded from Mona Island, with notes on their distribution, abundance, and host plants. Of this number of species, 197 were not previously known from the island. *Paradarnoides danforthi* n. sp. (Homoptera, Membracidae); *Paraprosoptropis* n. gen., *Paraprosoptropis monensis* n. sp. (Homoptera, Kinnaridae); *Flatoidinus pseudopunctatus* n. sp. (Homoptera,

Flatidae); *Ozophora octomaculata* n. sp. (Hemiptera, Lygaeidae); and
Ptychopoda monata Forbes, n. sp. (Lepidoptera, Geometridae) are
described.   The insect fauna of Mona Island is analyzed and discussed.

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EXPLANATION OF PLATES

PLATE I

1. *Paradarnoides danforthi* sp. nov.; lateral view. 2. Dorsal outline. 3. Frontal view of head. 4. Male genitalia. 5. *Paraprosoptropis monensis* sp. nov.; frontal view of head. 6. Lateral view of male genitalia. 7. Ventral view of female subgenital plate. 8. Dorsal view of head and thorax. 9. Tegmen.

PLATE II

10. *Flatoidinus pseudopuctatatus* sp. nov.; dorsal view of head and thorax. 11. Frontal view of head. 12. Ventral view of female genitalia. 13. Ventral view of male genitalia. 14. *Melormenis antillarum* Kirkaldy; ventral view of male genitalia. 15. *Flatoidinus pseudopunctatus* sp. nov.; lateral view of male genitalia. 16. *Colpoptera flavifrons* Osborn; lateral view of male genitalia. 17. *Melormenis antillarum* Kirkaldy; lateral view of male genitalia. 18. *Petrusa marginata* Brunnich; male genitalia of dark form. 19. Male genitalia of pale form. 20. *Ozophora octomaculata* sp. nov.; dorsal view.
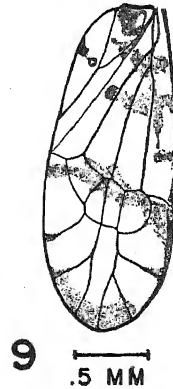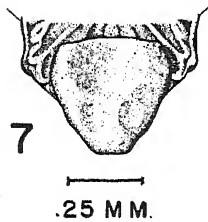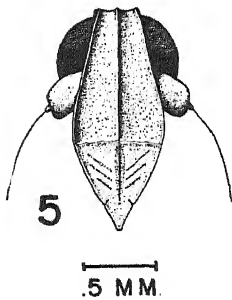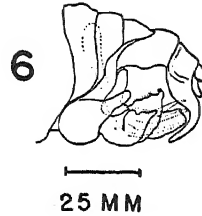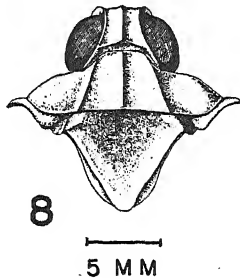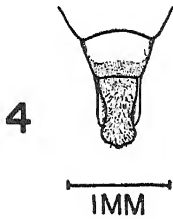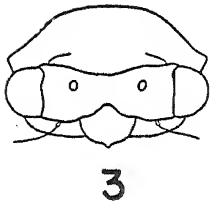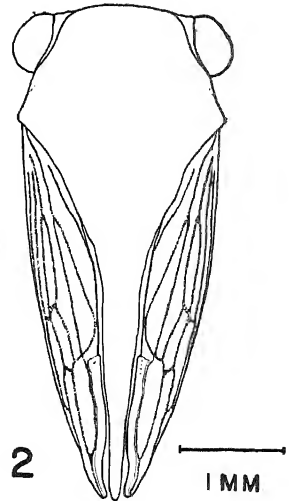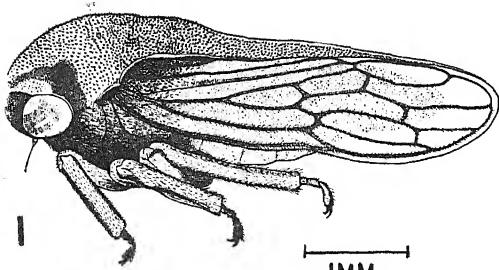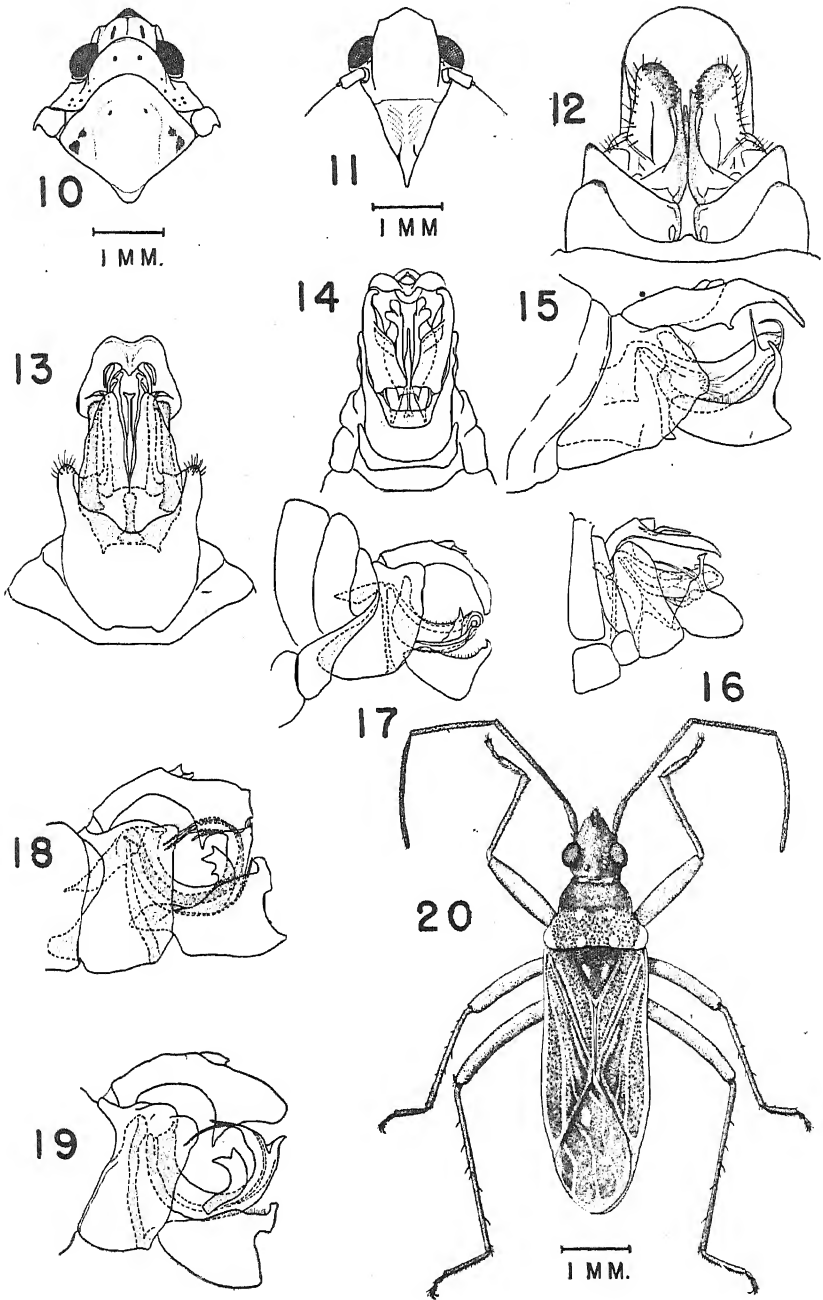
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(Ramos: Insects of Mona Island (West Indies))

THE CONTROL OF RHIZOCTONIA DAMPING-OFF OF PEPPER AND EGGPLANT IN PUERTO RICO

By LUIS A. ALVAREZ GARCÍA

TABLE OF CONTENTS

INTRODUCTION

Serious outbreaks of damping-off of vegetable crops have frequently been reported from almost every locality in the Island. The disease is more likely to occur during periods of heavy rainfall, when conditions of soil moisture and temperature are unsuitable for proper seedling development. Farmers sometimes complain of poor viability of seed when it is apparent that the low percentage of germination is due to pre-emergence failure caused by damping-off organisms. Heavy damping-off losses have occured in seed and plant beds of pepper and eggplant. In many instances, several thousand seedlings of these vegetables have succumbed to the disease. The disease has appeared in naturally contaminated soils as well as in artificially recontaminated, steamed or formaldehyde treated, soils.

Damping-off of vegetable crops in Puerto Rico has been ascribed by various workers to attacks by fungi of the genera *Pythium*, *Phytophthora*, *Phomopsis* and *Rhizoctonia*.

Nolla (10) found *Phomopsis vexans* (Sacc. & Syd.) Harter responsible for damping-off, blight and fruit rot of eggplant. *Pythium debaryanum* Hesse and species of *Phytophthora* were reported causing damping-off of tomato, pepper and eggplant.

Matz (7), in a study of the *Rhizoctonias* of Puerto Rico, reported isolates from beet, carrot, celery, tomato, citrus, eggplant, lettuce, corn, pepper, celeriac, banana, field pea, Natal plum (*Carissa grandiflora*), bean, "yautía" (*Xanthosoma* sp.) and hollyhock. No attempt was made to establish pathogenicity. The Rhizoctonia were studied morphologically and physiologically and cataloged by species, i. e., *R. microsclerotia*, *R. dimorpha*, *R. macrosclerotia*, *R. grisea*, *R. solani*, *R. ferrugena*, *R. pallida*, *R. alba* and *R. melongena*.

Tucker (16) reported strains of *Phytophthora capsici* Leonian, *P. palmivora* Butler and *P. parasitica* Dast. causing damping-off of tomato and eggplant. In Puerto Rico *P. capsici* has been found attacking only peppers.

Other organisms might possibly be associated with damping-off of pepper, tomato and eggplant seedlings in seed and plant beds in Puerto Rico. Aside from Nolla's (11) work on the control of damping-off of tomato, pepper and eggplant caused by *Pythium debaryanum*, *Phytophthora nicotiana* and *Phomopsis vexans*, the latter attacking only eggplant, very little attention has been given to the serious matter of controlling damping-off of vegetables in Puerto Rico.

Fungicides for seed and soil treatments have been tested and recommended in other countries to minimize losses due to damping-off of vegetables and other crops. It is a well-known fact that damping-off organisms

react differently to fungicidal treatments. This depends not only upon their specificity for toxic chemicals, but also upon variations of soil composition and climate. The occurrence of different physiological strains of fungi is also recognized. These factors account for the apparent discrepancies in effectiveness of fungicides for the control of certain plant diseases.

The present paper, therefore, treats of essential information regarding the occurrence of a damping-off of pepper and eggplant in seed and plant beds in Puerto Rico, the causal agent, the symptoms produced on infected seedlings, the life history of the pathogen, the influence of environmental conditions on damping-off, and the effect of seed and soil treatment with fungicidal chemicals for control of the disease.

<div align="center">CAUSAL ORGANISM</div>

During the summer and fall rainy season of the years 1941, 1942 and 1943, the Genetics Department of this Station was confronted with a serious case of damping-off of pepper (*Capsicum annuum* and *C. frutescens*). Several hundred seedlings obtained from crosses of the hot Mexican pepper known as "Cuaresmeño" and the variety "California Wonder", grown in flats in steamed soil (15 pounds for 2 hours), or formaldehyde treated (1 part to 40 of water), were completely lost due to damping-off. The soil used in every instance was a mixture of three parts of alluvial clay loam soil and one part of "cachaza", decomposed filter press cake from the sugar mills. Cultures in agar plates were made by planting pieces of infected tissues of pepper seedlings showing symptoms of damping-off. Tissue plantings were also made from diseased eggplant varieties "Rosita" and "Puerto Rican Beauty" seedlings found in seed and plant beds. Tissue plantings of diseased tomato, pepper and eggplant seedlings from the field were made during the course of the two years.

In a great majority of plates a rapidly growing fungus with coarse, septate, and branching mycelium, was obtained. The mycelium turned slightly brownish or dark-brown with age, grew irregularly, and formed large, aerial, coriaceous masses of sclerotia in culture. The characteristics of the organism in culture and in plants indicate a strain of *Rhizoctonia solani* Kühn.

The general cultural characters of the isolates from pepper and eggplant seedlings conformed very closely with those already described by Matz (7) for *R. solani*. Several *Fusaria* were also obtained on poured plates and were isolated in pure culture. Fifty isolates of *R. solani* were compared morphologically and physiologically, and there were no apparent differences among them. Pure cultures of *R. solani* and other organisms were obtained by spore and hyphal-tip isolations and were labelled R-1,

R-3, R-50, F-1, F-2, F-15; indicating *Rhizoctonia* and *Fusarium*, respectively.

ECONOMIC IMPORTANCE AND DISTRIBUTION

The widespread occurrence of *Rhizoctonia* damping-off and the fact that the pathogen can live indefinitely in the soil, makes this a very serious disease of vegetable crops in Puerto Rico.
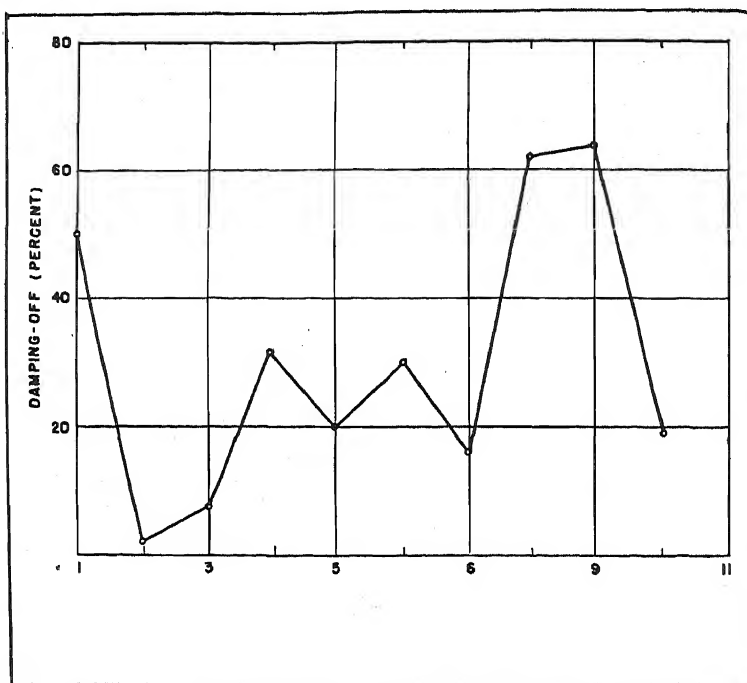
FIG. 1. Distribution of *Rhizoctonia solani* in various fields in Station ground

Damping-off in seed and plant beds has been very serious and 100 per cent losses have frequently been reported.

Preliminary observations of the incidence of the disease in pepper and eggplant seedlings have revealed a high degree of soil infestation of *Rhizoctonia* sp. in various fields at the Station.

The general distribution of these organisms in various fields is shown in graph No. 1. Fig. 1.

PATHOGENICITY

Eight isolates of *Rhizoctonia solani* from different seedbeds were tested for pathogenicity. Four *Fusaria* were similarly selected for comparative tests with the *R. solani* isolates.

Four-inch pots filled with a soil mixture of three parts alluvial, clay loam soil and one part of "cachaza" were steamed for two hours at 15 pounds pressure. Soon after cooling, the pots were arranged in 10 blocks of 13 pots each, on a cement table inside the plant pathology greenhouse. Pots of each block were numbered 1 to 13 at random, thus allowing 10 replicates for each culture.

Each corresponding pot of each block was infested with a corresponding culture. This was accomplished by taking one inch of soil from each of the 10 corresponding pots, mixing the soil in a steam sterilized, enameled pan; and adding to the soil a mixture of small pieces of mycelium and sclerotia from a mycelial mat 10 cm. in diameter. One separate culture of *R. solani* or one of *Fusarium* sp. was used in each soil treatment. The mycelial mat was obtained by growing the organisms separately in Coon's solution. The *Rhizoctonia* and the *Fusaria* grew well in this medium, the former producing abundant sclerotia and the latter abundant conidia. The mycelial mat of each culture was macerated with sterile sand in a sterile mortar thus obtaining a uniform mixture of the soil and macerate. One inch of the infested soil mixture was added to each corresponding pot in each block so that a uniform distribution of inoculum resulted.

Control pots were treated similarly but were not infested; sand only was added. Thirty eggplant seeds of the variety "Rosita" were sown in each replicate pot, totalling 300 seeds per treatment. The seed was sown one half inch deep and watered immediately. The rapid evaporation of water in clay pots necessitates daily watering. Records of germination, pre-emergence and post-emergence failures were taken daily for a period up to 15 days after germination. Damping-off occurred and killed most seedlings within the first three to five days after emergence (table 1).

The results obtained show that all *R. solani* isolates from eggplant and pepper seedlings, causing damping-off in seedbeds at the Station, are virulent and perhaps belong to the same strain. No significant differences existed among them, though there existed a marked difference in pathogenicity between the *R. solani* isolates and those of *Fusaria*. The results also show that the latter organisms are of no apparent importance in producing damping-off in pepper and eggplant. Tomato seedlings showed marked resistance to *Rhizoctonia*.

Reisolations from each corresponding group of 10 replicates of all damped-off seedlings yielded, in every instance, the fungus *R. solani*.

THE DISEASE

Post-emergence symptoms of damping-off of pepper and eggplant were characterized by the appearance of water-soaked areas on succulent stems at the soil level. The affected areas became black, necrotic, shrunken, and
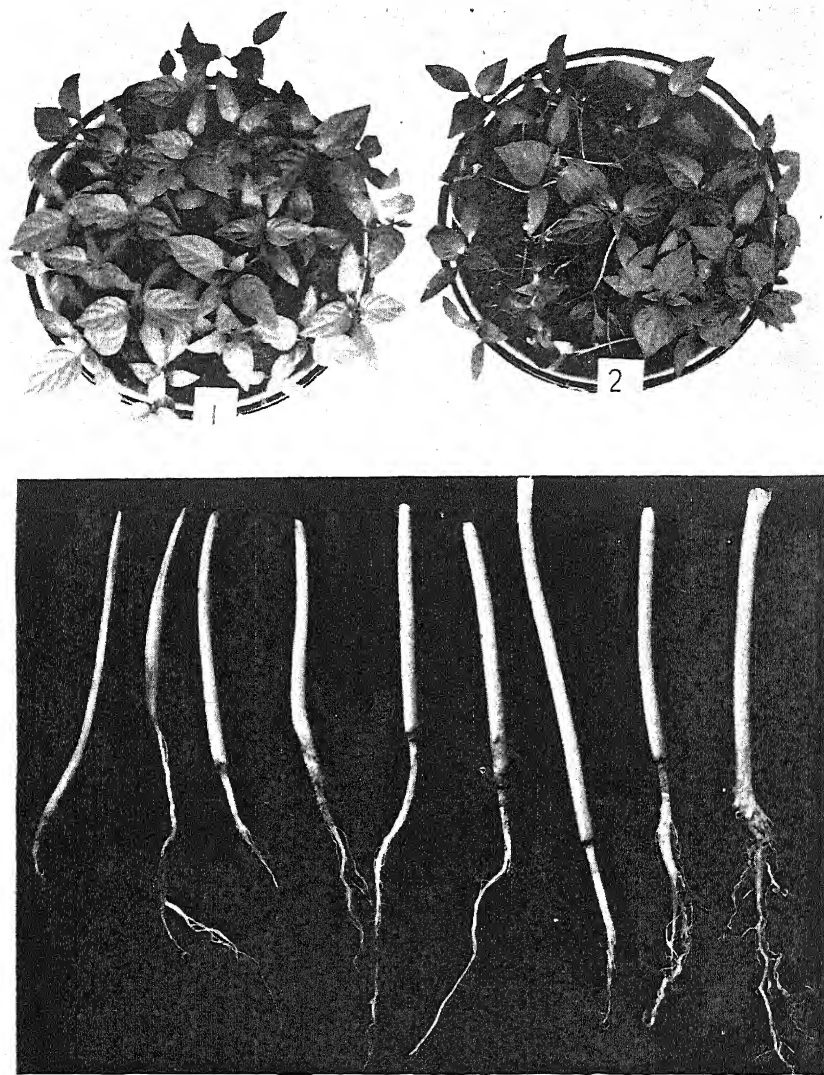
FIG. 2. Pepper seedling grown in the greenhouse on (1) left, soil artificially contaminated with *Rhizoctonia solani* (2) right, on steamed soil (3). Pepper seedlings grown on contaminated soil showing collar rot injury due to infection with *Rhizoctonia solani*.

the plants toppled over and dried up. The symptoms appeared almost immediately after emergence of seedlings, or within the first week of growth, when the tissues were succulent and more susceptible.

TABLE 1

Pathogenicity trial with single-hyphal-tip and monosporial cultures of fungi upon eggplant in steamed soil. Three hundred eggplant seeds* variety "Rosita" were sown for each treatment

Fungus	Culture number	Damping-off—failures				Total failure§	
		Pre emer-gence†	Pre emer-gence‡	Pre emer-gence	Post emer-gence		
		number	*number*	*per cent*	*per cent*	*number*	*per cent*
Rhizoctonia solani	R-1	200	40	85	100	240	100
Strains	R-2	189	40	84	79	229	95
"	R-3	167	39	69	50	196	81
"	R-7	215	25	89	100	240	100
"	R-9	195	39	81	86	234	97
"	R-11	199	22	82	53	221	92
"	R-15	222	17	92	94	239	99
"	R-41	211	20	91	78	231	96
Fusarium spp.	F-1	30	5	12.5	2.4	35	14
	F-7	19	0	8	0	19	8
	F-9	10	7	4.5	3	17	7
	F-13	17	3	7	1.2	20	8.5
Control	¶	5	—	2	0	5	2

* Three hundred seeds 80 per cent germination in control.

† Pre-emergence failure, per cent $= \dfrac{240\text{-seed germinated}}{240}$.

‡ Post-emergence failure, per cent $= \dfrac{\text{Post-emergence failure}}{240\text{-seed germinated}}$.

§ Total failure, per cent $= \dfrac{\text{Pre} \div \text{post-emergence failures}}{240}$.

¶ Not inoculated.

Analysis of variance for mean-square difference between organisms, according to data for table 1

	Degrees of freedom	Sum of squares	Mean square	F¹
Total..........................	129	14,404		
Blocks........................	9	8,479		
Treatment.....................	12	3,913	326	12**
Error..........................	108	2,012	18.6	

** Significant at the 1 per cent point

Pepper and eggplant were very susceptible during the first week after emergence. There was a marked resistance with increasing age of seedlings. Under field conditions, damping-off of pepper and eggplant was

characterized by the blackening and shrinking of stem tissues at the soil level and extending up the stem, never down to the roots.   In very wet soils, or during periods of heavy rainfall, the fungus was observed as a white web on the necrotic stem lesions.   No sclerotia, however, were found on infected tissues of diseased plants (fig. 2. photo of diseased pepper seedlings).

TEMPERATURE

Temperature Relations of Rhizoctonia Solani

Cultures of *R. solani* were grown on potato two per cent dextrose agar and incubated at different temperatures.   Four duplicates were made for
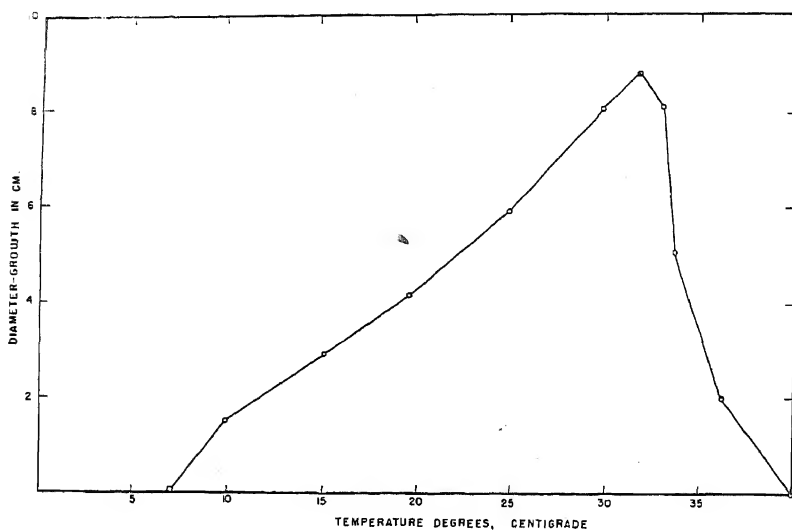
FIG. 3. Growth of *Rhizoctonia solani* in 90 hours at various temperatures

each incubation temperature.   Measurements of two diameter at right angles to each other for each colony were taken daily and increments in diameter of growth during a three day period also were taken.   The average diameter for each set of four plates was recorded.   Maximum growth appeared to be at 28–32°C., and a sudden decrease occurred at 34°C.   Very slow growth resulted below 20°C.

High temperatures were found favorable to the occurrence of *Rhizoctonia* damping-off.   Pathogenicity tests conducted in greenhouses (22–40°C.) showed significant results as to the amount of damping-off caused by strains of *Rhizoctonia*.   The incidence of the disease, therefore, seems to substantiate the published statements that strains of *Rhizoctonia* cause damping-off at high temperatures.   In our tests, the average temperature was approximately 28°C.

ACIDITY

Effect of pH of Substrate on Growth

To determine a possible correlation of soil acidity with the development of the organism, *R. solani* was grown on potato two per cent dextrose agar adjusted to varying pH value. Lots of four plates, each lot adjusted to a different pH value, were separately planted with mycelial disks, approximately 0.5 cm. in diameter, of the fungus and set aside at 28°C., in the dark. Twenty-four hour increments in diameter-growth of the colonies were recorded. These measurements showed that the organism grew favorably at pH values near neutrality or slight alkalinity.
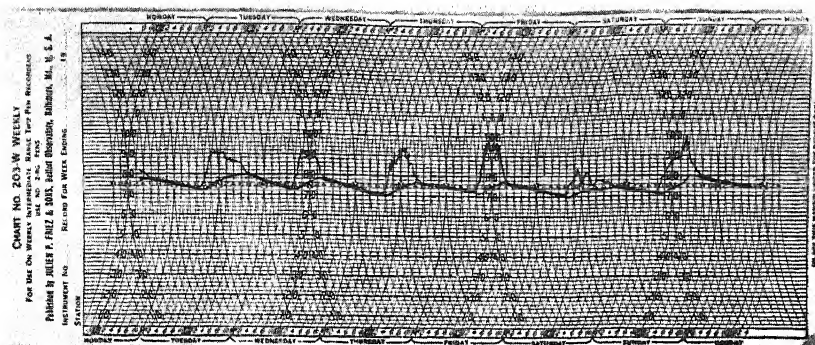
FIG. 4. Graph showing weekly fluctuation of soil and air temperatures in the greenhouse. Smooth curve represents soil temperature and broken curve air temperature.

The growth of *R. solani* species at varying pH values of substrate has been determined by many investigators and there is much divergence among the observations reported (6, 13). The variations reported for the optimum acidity for growth may be explained on the basis of diversified strains of the fungus.

Acidity and Damping-off

No experiments were conducted to determine the effect of soil acidity on the occurrence of damping-off. It was observed that all the pH determinations of soil samples taken from seed and plant beds where the disease had been serious, ranged from pH 5.00–6.50. Observations reported for similar investigations have shown that damping-off caused by *Rhizoctonia* spp. is abundant in either acid or alkaline soils (3, 13).

Soil mixtures of three parts of alluvial, clay loam soil and one part of "cachaza" are generally used in the Station in seed and plant beds. They
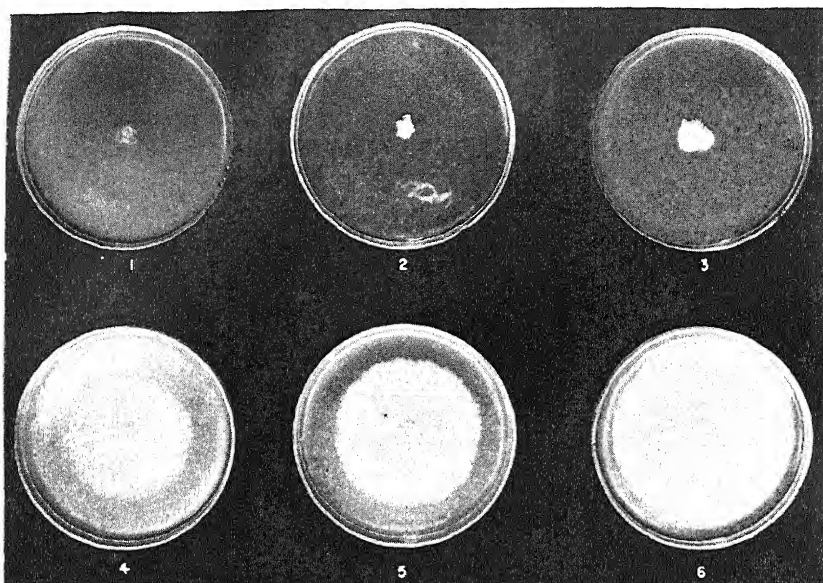
FIG. 5. Growth of *Rhizoctonia solani* in 90 hours at 28°C., on potato dextrose agar adjusted to varying pH's values of 1.0, 1.2, 1.5, 5.49, 601 and 7.08.
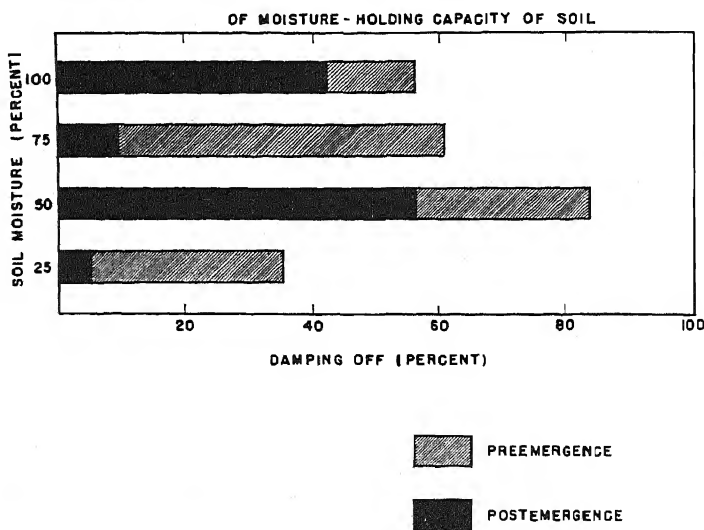
FIG. 6. Damping-off of eggplant seedling caused by *Rhizoctonia solani* at various percentages of the moisture-holding capacity of the soil.

have pH values approximately neutral. In a great majority of cases the reaction has been a pH of 6.54.

MOISTURE

Soil Moisture

It is claimed by many authors (2, 3, 13) that the percentage of soil moisture greatly influences the development of damping-off. High moisture content of the top soil is considered more important than the total percentage of water in the soil.

Rainfall: Agricultural Experiment Station—Rio Pedras, P. R.
From January 1932 to December 1943
Data compiled by the Department of Agronomy

Year	Janu-ary	Feb-ru-ary	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	Aver-age 12 yrs.
1932	6.03	0.65	1.16	1.65	13.03	8.96	7.12	7.55	11.94	10.63	4.30	4.43	77.45	
1933	3.20	1.69	5.23	2.99	7.00	4.02	5.98	10.18	13.36	6.03	5.21	5.59	70.48	
1934	5.72	1.88	3.34	1.12	2.34	6.05	7.14	4.70	6.29	6.16	6.08	12.85	63.67	
1935	2.80	6.10	1.73	1.66	7.34	5.29	6.98	7.45	6.00	6.62	3.86	1.97	57.80	
1936	1.58	1.03	.88	1.87	16.11	5.49	10.42	8.37	8.92	6.85	2.38	8.61	72.51	
1937	17.39	1.08	.47	1.54	2.08	4.90	9.25	12.53	9.17	5.42	6.32	4.14	74.29	
1938	3.89	3.23	3.45	2.20	5.14	17.55	4.56	6.40	5.75	7.47	10.20	5.39	75.23	
1939	4.01	3.85	3.79	2.78	13.04	3.32	8.48	9.60	8.23	8.85	12.83	3.65	82.43	
1940	4.21	4.24	.99	9.67	12.23	4.84	5.25	4.44	2.92	6.12	7.47	3.64	66.02	
1941	3.13	.73	1.78	5.71	16.45	11.24	9.80	8.90	7.25	5.72	7.61	3.83	82.15	
1942	1.63	2.32	2.18	6.89	5.39	4.42	10.07	7.60	10.32	8.27	9.78	4.13	73.00	
1943	15.55	4.95	4.86	6.74	7.22	8.30	8.66	14.12	4.00	7.81	.85	2.84	85.05	73.34

Data from the United States Department of Agriculture Weather Bureau
Average monthly and annual rainfall

	Ele-va-tion	No. of years	Janu-ary	Feb-ru-ary	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	An-nual
	feet														
Río Piedras	75	36	4.96	3.28	3.45	4.52	6.90	6.10	7.69	7.90	8.27	6.63	6.99	6.33	73.02

Sets of four one-gallon enameled pots filled with the above-mentioned soil mixture (pH 6.54) were steamed for two hours at 15 pounds pressure, and soon after cooling were placed on cement tables in the plant pathology greenhouse. The weighing method was used to determine the effect of soil moisture on damping-off. No attempt was made to reduce the evaporation of water from the surface layers of the soil. The purpose was to conduct the experiment by duplicating, as nearly as possible, the conditions in seed and plant beds where the surface layers of the soil are free to evaporate

water at all times.   The practical aspect of the problem has primarily been considered.

The percentage of damping-off was very high.   The surface layers of the soil were saturated in all cases because water was added daily to replace that lost by evaporation.

The results revealed that the amount of soil moisture is very important, provided that the water is near the surface layers.   In all probability, different results could have been obtained if water had been supplied from the bottom by capillarity.   Farmers apply water by means of sprinklers and use capillary irrigation in only a few instances.

TABLE 2

Varietal behavior of Capsicum annuum and C. frutescens when grown on Rhizoctonia solani artificially infested soil

Number	Variety of pepper	Amount viable seed sown	Damping-off failure				Failure	
			Pre-emer-gence failure	Post-emer-gence failure	Total			
					Pre-emer-gence	Post-emer-gence		
			number	*number*	*per cent*	*per cent*	*number*	*per cent*
1	Early Giant	800	415	83	52	22	498	62
2	Windsor A	800	400	175	50	43	575	72
3	Tabasco	800	242	177	30	32	419	52
4	King of the North	800	350	59	44	13	409	51
5	Red Chili	800	131	43	16	6	174	22
6	Maule's Red Hot	800	135	136	17	20	271	34
7	Sweet Meat Glory	800	114	57	14	8	171	21
8	Large Early Neapolitan	800	165	57	21	9	222	28
9	Large Red Cheyenne	800	70	140	9	19	210	26
10	Hungarian's Way	800	144	127	18	19	271	34
11	Chinese Giant	800	155	56	19	9	211	26
12	Fordhook	800	496	85	62	28	581	73
13	Large Chemy	800	85	40	11	6	127	16
14	Sweet Banana	800	301	55	38	11	356	45
15	World Beaters	800	63	82	8	11	145	16
16	Yellow Oskosh	800	66	43	8	6	109	14
17	Ruby King	800	341	81	43	18	422	53
18	Bull Nose	800	152	96	19	15	248	31
19	California Wonder	800	247	97	31	18	344	43
20	Large Bell Hot	800	242	54	30	10	296	37
21	Sunny Broock	800	338	44	42	10	382	48

$$\text{Per cent pre-emergence} = \frac{\text{seed sown} - \text{seed germinated}}{800}.$$

$$\text{Per cent post-emergence} = \frac{\text{post-emergence failure}}{\text{seed sown} - \text{pre-emergence failure}}.$$

Summary of analyses of variance for data of table 2

Comparison of failures	DF	Sum of squares	Variance	F.
Post-emergence (per cent basis) failure				
Total........................	167	76,000		
Blocks	7	4,447		
Varieties.....................	20	7,337	366.8	0.80
Error........................	140	64,216	458.7	
Pre-emergence failure				
Total........................	167	121,875.9		
Blocks.......................	7	20,249.8		
Varieties.....................	20	41,549.6	2,077.5	4.84*
Error........................	140	60,076.4	429.1	
Total failure				
Total........................	167	132,826		
Blocks.......................	7	12,286		
Varieties.....................	20	36,358	1,817.9	3.02*
Error........................	140	84,182	601.3	

* Significant at the 1 per cent level.

Reports of the Weather Bureau for the last 36 years show that eight months in a year have a monthly average rainfall of more than 6 inches. The heavy nature of the soil around Río Piedras and the abundant rainfall favor the development of damping-off.

<div align="center">VARIETY TEST</div>

Testing Pepper Varieties for Resistance to Damping-off

Twenty-one varieties of sweet and hot peppers *Capsicum annuum* and *C. frutescens* were tested for resistance to damping-off caused by *Rhizoctonia solani*. One hundred seeds of each variety were sown in rows in blocks in a pan and replicated eight times, a total of 800 viable seeds per variety, considering in each case the percentage of germination in sterilized soil. The soil used was the above-mentioned mixture with "cachaza," to which macerates of sclerotia and mycelium of pure cultures of the organism were added. The seed was sown ½ inch deep and the soil was watered immediately after sowing with a sprinkler, until saturation resulted. The pan was drained at the bottom to prevent water logging.

Diseased seedlings from each row were pulled up for tissue plating. *R. solani* constantly was associated with damping-off.

There were significant differences in susceptibility in the pre-emergence stage of development of the pepper varieties investigated. It is apparent,

therefore, that at this early stage of development some varieties are either more susceptible to attack by *R. solani* than others, or the possibility exists that even though an equal amount of seeds was sown, based on germination tests, they did not germinate according to expectancy.

Records obtained during the post-emergence stage demonstrated no differences in susceptibility of varieties tested. Analysis of pre- and post-emergence failures showed that some varieties were more susceptible than others. All varieties were equally susceptible to damping-off, if only the post-emergence failure was considered.

Analysis of variance of a varietal test with eggplant varieties "Rosita," "Puerto Rican Beauty" and "Black Beauty" showed no significant differences in susceptibility. Tomatoes were also attacked by the isolates of *R. solani*. Pritchard and Porte (12) found in a study of collar rot of tomatoes that a strain of *R. solani* caused only three per cent infection in seedbeds, while *Verticillium lycopersicii* and *Macrosporium solani* produced 64 and 75 per cent infection, respectively.

FUNGICIDAL TREATMENT

Effect of Seed and Soil Treatment in Controlling Damping-off

The value of seed and soil treatment for the control of damping-off of many vegetable crops has been well established. Seed and soil treatment is most effective in soils with a moderate "inoculum potential". The effectiveness of various fungicidal dusts recommended for seed and soil disinfection was tested for the control of damping-off of pepper and eggplant caused by *Rhizoctonia solani*. Both naturally and artificially inoculated soils consisting of three parts of alluvial, clay loam soil and one part "cachaza," pH 6.54, were used in the tests.

Two hundred four-inch pots were filled to one inch from the top with the above-mentioned soil mixture. The pots were steamed for two hours at 15 pounds pressure and soon after cooling were placed on sand beds in an insect-free insectary inside a greenhouse. The plan for the test was a random-block design consisting of 10 blocks of 40 pots each. Twenty of the 40 pots of every block were filled with naturally infested soil. Pots not steamed were labelled with odd numbers from 1 to 39. Steamed pots were labelled from 2 to 40. Pots 2 to 40 were infested with equal proportions of macerates of mycelial mats of *Rhizoctonia solani* grown in Coon's liquid medium.

Seed Treatment

Five hundred seeds in each case were treated separately with one chemical, except groups 19 and 20 which served as checks. Sufficient chemical

dust was added to every lot of seed to cover its surface. The surplus chemical was separated by means of a fine-mesh-wire screen.

Twenty-five seeds of each treatment were sown in corresponding pots in each block. The pots were watered with sterile water immediately after sowing the seed.

Analysis of the results indicated a very definite effect upon the type of infestation and the effectiveness of the fungicides. Significant differences were obtained among the fungicides tested when compared with either naturally or artificially infested soil. The mean square of variance corresponding to differences between fungicidal treatments and the type of infestation, the interaction of type and infestation, and their mean square for error, exceeded the one per cent level, indicating, therefore, the degree of effectiveness of the treatments.

Interpretation of Results Obtained from Analysis of Variance

Pre-emergence Failure:

On naturally infested soil: The least significant difference among treatments showed that the degree of damping-off during the pre-emergence stage of seedlings development was low, indicating a low "inoculum potential" of the soil. None of the fungicidal treatments tested seemed to promote a better germination of the seed because of either fungicidal or perhaps stimulatory effects. Statistically, these fungicides were equally ineffective when compared with the check, number 19. This can be interpreted on the basis of low "inoculum potential" of the soil which enabled the seed of all pots to germinate equally well.

However, "Spergon," "Arasan," "1155 H. H.," "2% Ceresan" and "New Improved Ceresan" apparently were injurious to the seed. This is indicated by the low percentage of germination which statistically is significant when compared with the check, number 19.

On artificially infested soil: "Semesan," "Z-O," "Dipdust," "Cuprous Oxide," "Zinc Oxide" and "Coppercarb" were equally effective in controlling pre-emergence failure. The percentage of germination was statistically higher when compared with the corresponding number of seedlings emerging in the check, number 20.

"New Improved Ceresan," "1155 H. H.," and "2% Ceresan" again proved toxic to the seed, and reduced viability materially and statistically when compared with the check, number 20.

Post-emergence Failure:

On naturally infested soil: "Coppercarb," "Barbak D.," "Fermate" and "Dipdust" showed some harmful effects because the percentage of

failure was statistically higher than that obtained in the check, number 19 which was zero. Other treatments were equally ineffective in preventing post-emergence damping-off.

On artificially infested soil: "Spergon," "Cuprous Oxide," "1155 H. H.," "2% Ceresan," "Cupric Oxide" and "Barbak D." were equally effective in preventing post-emergence failure. The percentage of surviving seedlings was statistically higher than that obtained in the check, number 20. "New Improved Ceresan," "Semesan Jr." and "Dipdust" treatments were statistically inferior when compared with the check, number 20.

Pre- and Post-Emergence Failure:

On naturally infested soil: No fungicide caused statistically higher percentages of germination and development of emerged seedling than that observed in the check, number 19. "Spergon," "1155 H. H.," "2% Ceresan" and "New Improved Ceresan," however, reduced the germination and development of seedlings below that of the check, number 19.

On artificially infested soil: "Bayer Compound No. 1494" was effective in preventing post-emergence damping-off of seedlings, either before or after emergence of the seedlings. The effect was statistically higher than that obtained in the check, number 20. "New Improved Ceresan" demonstrated once more a toxic effect on the seed, as shown statistically in comparison with the check, number 20.

Soil Treatment

The same procedure used in preparing pots for testing the effectiveness of the seed treatment was followed, except that the soil and not the seed was treated with chemical dusts. All treatments were applied at the rate of 1.0 gram per square foot of soil, except that for zinc oxide, which was applied at the rate of 10.00 grams per square foot of soil. The soil for each treatment was separately and evenly mixed with one chemical. After a thorough mixing, the infested soil was placed in 10 corresponding pots and one pot was distributed in each block.

Result of Analysis of Variance for Soil Treatment:

Pre-emergence Failure: On naturally infested soil: In accordance with results obtained from soil treatment, all fungicides were apparently equally ineffective in preventing damping-off, when compared statistically with the check, number 19. None of the treatments were either better or worse than the check. No treatment showed toxic effects upon the viability of the seed.

On artificially infested soil: All treatments were equally ineffective in preventing pre-emergence failure and none were better or worse than the

TABLE 3

Effect of seed treatment on pre-emergence, post emergence and total emergence failure of eggplant seedlings, variety "Rosita." Seed sown, 250. Soil infested with Rhizoctonia solani (isolated from eggplant seedlings)

Number	Trade name	Treatment	Manufacturing house	Damping-off Pre-emergence	Damping-off Post-emergence	Total Pre-emergence	Total Post-emergence	Total failure number	Total failure per cent
				num-ber	num-ber	per cent	per cent	num-ber	per cent
1	Arasan	Tetramethyl-	Bayer-Semesan Co.	25	0	10	0	25	10
2	"	thiurum-bisul-	"	81	138	32	82	219	88
		fide. 50%.							
3	Spergon	Tetrachloro-para-	U. S. Rubber Co.	67	18	27	10	85	34
4	"	benzoquinone	"	95	91	38	59	186	74
5	New improved	Ethyl-mercury-	Bayer-Semesan Co.	247	0	99	0	247	99
	ceresan	phosphate 5%							
6	" "		"	249	0	100	0	249	100
7	Fermate	Ferric-dimethyl-	Graselli Chem. Div.	32	20	13	9	52	21
8	"	di thio-carbamate	" "	82	108	32	64	188	75
9	Du Bary 1155	Ethyl-mercury-io-	Bayer-Semesan Co.	151	1	60	1	152	61
	H. H.	dide 5%.							
10	" "		"	173	31	69	40	204	82
11	Dipdust	Hydroxy-mercury-	Bayer-Semesan Co.	23	23	9	10	46	18
12	"	chloro-phenol-	"	45	171	18	83	216	86
		sulphate 6%							
13	Semesan	Hydroxy-mercury-	Bayer-Semesan Co.	23	0	9	0	23	9
14	"	chlorophenol.	"	21	161	8	70	182	73
		30%							
15	2% Ceresan	Ethyl-mercury-	Bayer-Semesan Co.	219	0	88	0	219	88
16	"	chloride. 2%	"	224	9	90	35	233	93
17	Z-O			22	0	8	0	22	9
18	"			30	141	12	64	171	68
19	Check			27	0	11	0	27	11
20	"			138	70	55	25	208	83
21	Coppercarb	Copper carbonate	Tennessee Copper	35	33	14	15	68	27
22	"	" "	Corp.	74	131	30	74	205	82
23	Cuprocide	Cuprous oxide	Rahn & Hass Co.	34	20	14	9	54	22
24	"	" "	"	93	80	37	51	173	69
25	ZN-O		Rahn & Hass Co.	27	0	11	0	27	11
26	"		"	73	106	29	60	179	72
27	Cupric oxide	Cupric oxide	General Chemical	33	7	13	3	40	16
28	" "	" "	Co.	123	38	49	30	161	64
29	Uspulun	Chlorophenol mer-	Bayer-Semesan Co.	61	2	24	1	63	25
		cury							
30	"	" "	"	144	85	58	80	229	92
31	Barbak D	Mercuric phenyl	Am. Cyanamid &	98	1	39	0	99	40
		cyanamid 6%	Chem. Co.						
32	"	"	"	161	35	64	39	196	78
33	Dupont no. 1		E. I. Dupont Co.	15	8	6	3	23	9
34	"		"	164	46	66	53	210	84
35	Cuprous oxide	Cuprous oxide	Mallinckrodt Co.	25	1	10	0	26	10
36	" "	" "	"	155	85	66	100	250	100
37	Semesan Jr.	Ethyl mercury phos-	Bayer-Semesan Co.	40	22	16	10	62	25
38	"	phate 1%	"	80	149	32	88	229	92
39	Bayer com-		Bayer-Semesan Co.	66	20	26	11	86	34
	pound no. 1494								
40	" "		"	147	74	59	72	221	88

(1) Odd numbers = naturally infested soil.

(2) Even numbers = artificially infested soil.

(3) Per cent calculation on the basis of 250 seeds that germinated in controls.

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Analysis of variance for table 3

Failures	Degrees of freedom	Sum of squares	Variance	F.
Pre-emergence				
Total...	399	26,094.1		
Blocks..	9	572.28		
Treatments...................................	29	18,060.2	463.08	21.78*
Kind of infestation.........................	1	2,475.0	2,475.0	116.46*
Types.....................................	19	6,923.0	364.0	17.12*
Infestation x types.........................	19	8,662.2	456.0	21.45*
Error...	351	7,461.62	21.25	
Post emergence (per cent basis)				
Total...	399	530,526		
Blocks..	9	6,880		
Treatments...................................	39	446,162	1,145.0	52.0*
Kind of infestation.........................	1	327,177	327,177	1,032.0*
Types.....................................	19	28,342	1,491.2	6.7*
Infestation x types.........................	19	1,563.27	8,227	37.3*
Error...	351	77,484	220.0	
Total failure				
Total...	399	35,301.5		
Blocks..	9	609.5		
Treatments...................................	39	22,511.3	577.2	16.6*
Kind of infestation.........................	1	13,294.1	13,294.1	38.3*
Types.....................................	19	5,498.4	289.3	8.3*
Infestation x types.........................	19	3,718.8	195.2	5.6*
Error...	351	12,180.7	34.7	

* Significant at the one per-cent level.

check, number 20; but "Spergon," "Dipdust," "Fermate," "New Improved Ceresan" and "Semesan Jr.," apparently reduced germination.

Result of Analysis of Variance for Total Failure: On naturally infested soil: "Spergon," "1105 H. H.," "Zinc Oxide," "Bayer Compound No. 1494," "Semesan," "Dipdust," "2% Ceresan," "Z-O," "Uspulun," "Fermate," "Dupont No. 1." "Cupric Oxide," "Coppercarb," "Arasan," "New Improved Ceresan," "Cuprous Oxide," "Barbak D.," "Cuprocide" and "Semesan Jr." were equally effective in controlling damping-off. None of the treatments was inferior to the check number 19.

On artificially infested soil: "Zinc Oxide" applied at the rate of 10 grams per square foot of soil was effective in preventing damping-off when compared statistically with the check, number 20. "Dipdust," "Cuprous Oxide," "Cuprocide," "Dupont No. 1," "Semesan," "Spergon," "2% Ceresan" and "1155-H. H." were equally ineffective and showed no significant difference to the check.

"Fermate," "Z-O," "Arasan," "New Improved Ceresan," "Coppercarb," "Cupric Oxide," "Uspulun," "Barbak D.," "Semesan Jr." and "Bayer Compound No. 1494" were statistically considered, equally inferior to the check.

TABLE 4

Effect of soil treatments on pre-emergence and post-emergence failure of eggplant seedlings, variety "Rosita." Seed sown, 250. Fungicides applied one week before sowing seed at the rate of 1.0 gram per square foot of soil. Soil naturally and artificially infested with Rhizoctonia solani (isolated from eggplant seedlings)

Number	Trade name	Treatment	Manufacturing house	Damping-off failures				Total failure	
				Pre-emergence	Post-emergence	Total			
						Pre-emergence	Post-emergence		
				number	*number*	*per cent*	*per cent*	*number*	*per cent*
1	Arasan	Tetramethyl-	Bayer-Semesan Co.	60	0	24	0	60	24
2	"	thiurum-bisul-	"	246	4	98	100	250	100
		fide. 50%							
3	Spergon	Tetrachloro-para-	U. S. Rubber Co.	44	17	18	8	61	24
4	"	benzoquinone	"	173	50	69	75	223	89
5	New improved ceresan	Ethyl-mercury- phosphate. 5%	Bayer-Semesan Co.	43	21	17	10	64	26
6	" "		"	200	45	80	90	245	98
7	Fermate	Ferric-dimethyl-	Graselli Chem. Div.	57	0	23	0	57	23
8	"	dithio-carbamate	of E. I. DuPont Co.	189	55	76	90	244	98
9	Du Bary 1155- H. H.	Ethyl-mercury-io- dide 5%	Bayer-Semesan Co.	27	0	11	0	27	11
10	" "		"	215	33	86	94	248	99
11	Dipdust	Hydroxy-mercury-	Bayer-Semesan Co.	56	3	22	2	59	24
	"	chlorophenol-sul- phate 6%	"						
12	Dipdust	Hydroxy-mercury-	"	178	47	71	65	225	90
	"	nitrophenol sul- phate 2%							
13	Semesan	Hydroxy-mercury-	Bayer-Semesan Co.	49	4	20	2	53	21
14	"	chlorophenol. 30%	"	74	162	30	92	236	94
15	Ceresan	Ethyl-mercury-	"	55	0	22	0	55	22
16	"	chloride. 2%	"	128	95	51	78	223	89
17	Z-O		"	46	10	18	5	56	94
18	"		"	151	98	60	99	249	100
19	Check	Check		66	163	26	89	229	92
20	"	"		93	108	37	69	201	80
21	Coppercarb	Copper carbonate	Tennessee Copper	43	39	17	19	82	33
22	Corona	"	Corp.	117	133	47	100	250	100
23	Cuprocide	Cuprous oxide	Rahn & Hass Co.	32	72	13	33	104	42
24	Yellow Copper oxide	"		73	150	29	85	223	89
25	Aaz	Zinc oxide	Rahn & Hass Co.	31	0	12	0	31	12
26	Speal	"		68	129	27	71	197	79
27	Cupric oxide	Cupric oxide	General Chemical	33	43	13	20	76	30
28	"	"	Co.	72	178	29	100	250	100
29	Uspulun	Chlorophenol mer-	Bayer-Semesan Co.	36	21	14	10	57	23
30	"	cury	"	125	125	50	100	250	100
31	Barbak D	Mercuric phenyl	Am. Cyanamid &	60	41	24	22	101	40
32	"	cyanamid 6%	Chem. Co.	132	118	53	100	250	100
33	Dupont No. 1		E. I. Dupont Co.	59	20	24	10	79	32
34	"		"	93	157	37	100	250	100
35	Yellow Copper oxide	Cuprous oxide	Mallinckrodt Co.	49	18	20	9	67	27
36	"	"	"	109	118	44	84	227	91
37	Semesan Jr.	Ethyl mercury phos-	Bayer-Semesan Co.	72	37	29	21	109	44
38	"	phate 1%	"	207	43	83	100	250	100
39	Bayer 1494		Bayer-Semesan Co.	46	0	18	0	46	18
40	"		"	130	119	52	99	249	100

(1) Odd numbers = naturally infested soil.

(2) Even numbers = artificially infested soil.

(3) Per cent calculation on the basis of 250 seeds that germinated in controls.

Analysis of variance for table 4

Failures	Degrees of freedom	Sum of squares	Mean squares	F.	Least square difference
					per cent
Pre-emergence					64.75
Total	399	34,566.76			
Blocks	9	5,308.96	589.74		
Treatments	39	22,773.76	583.94	31.61*	
Kind of infestation	1	526.2	526.2	28.48*	
Type	19	13,904.2	731.5	39.06	
Type x infestation	19	8,343.36	439.12	23.12	
Error	351	6,484	18.47		
Total Failures					18.65
Total	399	32,390.2			
Blocks	9	156.3			
Treatments	39	29,232.9	749.0	131*	
Kind of infestation	1	27,208.5	27,208.5	477.3*	
Type	19	1,587.85	83.5	14.4*	
Type of infestation	19	536.55	28.2	4.9*	
Error	351	2,001.9	5.7		

* Significant at the one per-cent level.

TABLE 5

Greenhouse toxicity test with eggplant seed "Rosita" dusted with fungicidal chemicals before sowing on steam-sterilized soil. Air temperature inside greenhouse 70–100° F. Relative humidity 40–60%. Soil saturated twice daily

No.	Chemical trade name	Total emergence from 250 seeds	
		number	*per cent**
1	New Improved Ceresan	101	43
2	Du Bary 1155-HH.	139	60
3	2% Ceresan	125	54
4	Check	232	100
5	Spergon	234	100
6	Arasan	209	90
7	Dipdust	232	100
8	Bayer 1494	196	84

* Calculated on the basis of 232 seeds germinated in control.

Analysis of variance for table 5

Emergence	D.F.	Sum of squares	Variance	F.
Total	79	2933		
Blocks	9	84	9.3	
Treatment	7	2037	291	25.9†
Error	63	712	11.3	

† Significant at the 1% level.

It is evident from the results analyzed that the soil treatment was effective in those soils with a low "inoculum potential".

Some fungicides are powerful poisons and under certain conditions might cause death of vegetable seeds. In these studies, 2% "Ceresan," "Du Bary 1155-H. H." and New Improved Ceresan showed this toxicity to eggplant seeds. The results of a toxicity test are shown in table 5.

The least square difference among dust treatments show that "New Improved Ceresan," "Du Bary 1155-H. H." and "2% Ceresan," were equally and significantly injurious to eggplant seed.

CONCLUSIONS AND SUMMARY

Damping-off of vegetable crops in Puerto Rico is of great economic importance. Studies of the causative agent or agents, the symptomatology of the disease, the host-parasite relationship, the distribution, epiphytology and saprogenesis were considered necessary before attempting to formulate pertinent measures of control for this particular problem.

It is apparent from the data obtained that *Rhizoctonia solani* and possibly other organisms are chiefly responsible for the damping-off losses of pepper and eggplant seedlings both in seed and plant beds and in the field.

Damping-off was serious during the first three to seven days after seedling emergence. Thereafter, the ability of the organism to cause damping-off diminished rapidly as the age and hardness of the tissues increased. The injury to recently emerged seedling was a soft, wet and dark rot of stems near the soil level, which soon spread upward and seldom downward. The infected seedlings toppled and finally died. In older plants, the infected stem tissues turned dark and became shrunken. Old plants withstood the disease much better than seedlings. Many plants in the field succumbed to the disease during periods of heavy rainfall.

The *Rhizoctonia* species responsible for the damping-off were very active under our climatic conditions, i.e., a high temperature ranging from 26 to 30°C. the year round, and a high soil and air moisture content. The high water-holding capacity of soils around Río Piedras, and the high rainfall of this locality are important factors for the development of the disease.

The organism grew well at varying pH values of the substrate, particularly at pH values approximately neutral. Considering that pH determinations of top layers of soil from various fields and soil mixtures (three parts of alluvial, clay loam soil and one part of "cachaza") were found to be more alkaline than a pH of 6.00, the presence of the parasite and the development of the disease would be expected in these soils.

The *Rhizoctonia* under consideration has not been observed to produce sclerotia in tissues of diseased pepper or eggplant. However, sclerotia are

produced profusely in culture media, particularly in Coon's synthetic liquid medium. Though it has not been possible to find sclerotia on infected plants, the occurrence of the organism in the soil is apparent from observations made repeatedly upon soil samples. Samples of soil mixtures taken from time to time have shown constantly the presence of the parasite.

Soil sterilization with steam for three hours at 15 pounds pressure was found very effective in preventing damping-off. Formaldehyde was especially effective at a concentration of one part to 20 of water. If precautions were not taken, damping-off was likely to appear in steamed and formaldehyde treated soils due to re-contamination. Many failures occurring in our seed and plant beds were attributed to re-contamination.

In view of the importance of rendering soil mixtures for pots, flats and plant beds free from damping-off organisms, and also in view of the impossibility for many farmers to practice steaming or treating the soil with formaldehyde because of its relatively high cost, several fungicidal dusts were tested for effectiveness in controlling damping-off.

Among the fungicides tested, "Semesan," "Z-O," "Dipdust," "Cuprous Oxide," "Coppercarb," "Zinc Oxide," "Semesan Jr.," "Arasan," "Fermate" and "Spergon" were found equally effective as seed disinfectants for the control of the pre-emergence phase of *Rhizoctonia* damping-off. The small dose used for seed treatment had no residual effect to control post-emergence damping-off. Considering that great losses result every year due to pre-emergence failure, diminishing pre-emergence damping-off is a great saving of time and money.

All treatments for soil disinfection proved equally ineffective in preventing post-emergence failure.

Analysis of total failure, however, showed the effectiveness of soil treatment with "Spergon," "1155 H. H.," "Zinc Oxide," "Bayer Compound 1494", "Semesan," "Dipdust," "2% Ceresan," "Z-O," "Uspulun," "Fermate," "Dupont No. 1," "Cupric Oxide," "Coppercarb," "Arasan," "New Improved Ceresan," "Cuprous Oxide," "Barbak D.," "Cuprocide' and "Semesan Jr." in preventing damping-off in naturally infested soil.

It is apparent that mercurial and copper fungicides have a decided fungicidal effectiveness as seed and soil treatments for controlling *Rhizoctonia solani*. Montieth and Harmon (8) obtained similar results in the case of brown patch of turf caused by *Rhizoctonia* spp. "Uspulun," "Semesan," "Germesan," "Corona 620" and "Corona 640" were found effective. These workers found that mercurials in the form of sulphate, oxide, chloride and nitrate were effective for controlling the disease. Mercurous chloride was the most effective and the most economical considering that

$\frac{1}{5}$ of a pound was as good as one pound of "Uspulun" or "Semesan". Thomas (15) found copper carbonate, mercuric bichloride and "Uspulun" effective at the rate of 1.0–3.0 grams; 0.5–1.00 grams; and 1.0–2.0 grams per square foot of soil, respectively, for controlling damping-off of tomato caused by *Phytophthora* spp. Nolla (11) found: 1) That soil drenching with a one to 50 formaldehyde solution, or applications of 4–4–50 Bordeaux mixture, were effective for controlling damping-off of eggplant caused by *Phomopsis vexans*, though formaldehyde was the most effective and economical. 2) Treatment of soil with corona copper carbonate, at the rate of four grams per square foot of soil, was effective for control of damping-off of tomato, pepper and eggplant caused by *Pythium debaryanum*. 3) Application of copper stereate, at the rate of eight grams per square foot, was found ineffective for control of *Phytophthora parasitica*, but apparently controlled *Pythium debaryanum*. 4) Bayer dust and "Uspulun" were ineffective for controlling damping-off caused by *P. parasitica* and *P. debaryanum*. 5) Two applications of Bordeaux (4–4–50 and 5–5–50 strength at the rate of one half gallon per square foot of soil) were effective in controlling *P. parasitica* and *P. debaryanum*, but were ineffective after damping-off has appeared in seedbeds. 6) Uspulun and Bayer dust were found injurious to tobacco seedlings and ineffective for controlling damping-off. 7) Copper sulphate solution (4–5 pounds in 50 gallons of water) was ineffective at the rate of one half gallon per square foot of soil. 8) Effectiveness of copper fluorosilicate was questionable. 9) Acetic acid (1.0 and $\frac{1}{2}$ per cent solutions) applied at the rate of one half gallon per square foot of soil, did not prove effective for controlling *P. parasitica* and *P. debaryanum*.

These investigations showed that damping-off is a complex problem and many organisms are involved. A combination of control methods appeared, therefore, necessary to assure the destruction of the various pathogens. Steam and formaldehyde are the best methods of soil sterilization. However, our experience has shown that great care has to be exerted if re-contamination of the soil is to be avoided. Bordeaux mixture 4–4–50, applied during the first week after the seedlings emerge, should accompany soil sterilization in order to minimize the chance of damping-off due to reinfestation of the soil.

The Bordeaux was applied at the rate of one half gallon per square foot of soil.

Soil sterilization with steam or formaldehyde are practices that many of our farmers are in no position to use at the present time. It would be very convenient, therefore, to control damping-off by the use of seed and soil treatments with fungicidal dusts already on the market.

Damping-off of tomato, pepper and eggplant in Puerto Rico is, so far as our knowledge is concerned, caused by species or strains of *Phomopsis*, *Phytophthora*, *Pythium* and *Rhizoctonia*.

Some mercurial and copper compounds have demonstrated their effectiveness for controlling these damping-off organisms in Puerto Rico. The possibility of using one or perhaps a combination of fungicides for controlling these types of damping-off appears to be a very satisfactory control measure.

RESUMEN EN ESPAÑOL

La podredumbre de semillas y plantitas de tomates, pimientos y berenjenas en los semilleros, ya en cajones o ya en el campo, presenta con harta frecuencia un carácter alarmante en nuestro ambiente. Esta podredumbre, conocida comúnmente por "salcocho," representa uno de los problemas más serios que confronta el hortelano.

En Puerto Rico se ha encontrado que varios organismos de los géneros *Pythium*, *Phytophthora*, y *Phomopsis* son responsables de enfermedades de esta clase. Recientemente apareció en semilleros de berenjenas y pimientos en la Estación Experimental una endofitotia de salcocho. Se pudo comprobar que dicho salcocho era causado por el ataque de un hongo cuyas características morfológicas y fisiológicas lo catalogan como una raza de *Rhizoctonia solani* Kühn.

La siembra de semillas de pimientos y berenjenas en tiestos con muestras de tierra representativas de varios campos de la Estación Experimental revelaron claramente la diseminación del hongo mencionado y la gran infestación de dichos terrenos. Este parásito es extremadamente agresivo, atacando las plantitas mucho antes de emerger del terreno y también después de haber emergido.

Los síntomas del salcocho aparecen durante la primera semana de surgir las plantitas, cuando los tejidos del tallo son más susceptibles al ataque del organismo. La enfermedad disminuye gradualmente según van envejeciendo y endureciéndose los tejidos del tallo. Los síntomas se caracterizan por la aparición de manchas acuosas en los tejidos del tallo, a flor de tierra. Luego ennegrecen estos tejidos infectados, síguele un constreñimiento de la parte afectada, y la planta termina por acostarse sobre el terreno del semillero y secarse.

Puede colegirse por lo expuesto anteriormente, que las pérdidas causadas por la podredumbre o salcocho en el estado pre-emergente, como también después de emerger la semilla, son considerables en muchos casos.

La enfermedad aparece más frecuentemente en terrenos húmedos debido a su naturaleza impermeable o por estar mal desaguados, o bien por factores climáticos, principalmente abundante precipitación pluvial. El pH del

terreno, según muchos investigadores, es factor de importancia para el curso de la enfermedad, particularmente en aquellos terrenos en que su pH fluctúa entre 6.00 y 7.00 o es ligeramente alcalino. Dentro de nuestro ambiente, en que prevalece una temperatura bastante alta durante todo el año y que fluctúa entre los 28°C y 30°C., y en que la precipitación pluvial es abundante—en Río Piedras en este caso en que nos ocupamos es de aproximadamente 6 pulgadas por mes—la patografía de la enfermedad se manifiesta con rapidez. Varias pruebas fisiológicas realizadas con este hongo demostraron su gran capacidad para crecer rápidamente en substratos húmedos, de pH variable, entre los 4.44 y 7.15, y a temperaturas entre los 7°C. y 34°C. En general, el hongo crece más rápidamente en un pH alrededor de 7, y a temperaturas entre 28 y 30°C. Este organismo puede crecer en infinidad de medios azucarados. En la disolución de Coon el patógeno produjo abundante micelio y esclerocios.

En la tabla número 1 del texto en inglés, se demuestra biométricamente que los diferentes cultivos de *Rhizoctonia*, obtenidos de pimientos y berenjenas enfermas en varios semilleros, son igualmente virulentos. Esto hace suponer que todos estos cultivos correspondan a una misma raza del patógeno.

Otros organismos del género *Fusarium* fueron también aislados. En las pruebas de patogenia estos organismos no revelaron en momento alguno estar relacionados con el desarrollo del salcocho, comportándose, por lo tanto, como meros saprófitos. Aislado ya en cultivo el patógeno, conocida su morfología y fisiología, y su comportamiento dentro de nuestras condiciones ambientales, se procedió inmediatamente a probar patogénicamente un sinnúmero de variedades de pimientos y berenjenas con el fin de determinar el grado de susceptibilidad al patógeno. En la tabla número 2 del texto en inglés se demuestra en su análisis que todas las variedades de pimientos y ajíes son igualmente susceptibles al salcocho. Las pruebas con variedades de berenjenas "Rosita," "Puerto Rican Beauty," "Pompadour" y "Black Beauty" revelaron que todas estas variedades son igualmente susceptibles a la *Rhizoctoniosis*.

En vista de que las variedades de pimientos y berenjenas no son resistentes a esta enfermedad, se hicieron varias pruebas con un sinnúmero de productos químicos con el fin de ver si alguno o varios de ellos resultaban efectivos en combatir la enfermedad.

En primer término, se dudó de la eficacia de esterilizar con vapor el terreno por dos horas a 15 libras de presión y también con formalina en dilución de una parte por 20 de agua, y aplicada esta dilución a razón de un galón por pie cuadrado de terreno. Los resultados demostraron la efectividad de estos tratamientos en evitar el salcocho.

El tratamiento del terreno con caldo bordelés 4-4-50 a razón de medio

galón por pie cuadrado de semillero, y con sulfato de cobre en la proporción de cuatro libras por 50 galones de agua y a razón de un galón por pie cuadrado de terreno, fué bastante efectivo, aunque no tan eficaz como los métodos anteriormente indicados. El caldo bordelés aplicado en la fórmula arriba indicada, después de germinar las semillas, contribuyó grandemente a evitar la propagación del patógeno a partir de brotes esporádicos de salcocho en los semilleros. Los mejores resultados en caso de brotes se obtuvieron aplicando a la zona infestada formalina en la dilución indicada.

Dada la imposibilidad de muchos agricultores de usar los métodos de desinfección del terreno mencionados anteriormente por ser bastante costosos, se resolvió probar varios polvos fungicidas de precios módicos actualmente en el mercado. Las pruebas tenían por objeto determinar el comportamiento de estos fungicidas dentro de nuestras condiciones climáticas, ya que se sabe que estos productos químicos varían en su efectividad dentro de diferentes condiciones ambientales y de acuerdo con la naturaleza de la enfermedad. Las pruebas se hicieron en tiestos llenos de tierra naturalmente infestada con *Rhizoctonia*, o, y con tierra infestada artificialmente con el mencionado organismo. De este modo se establecieron dos experimentos paralelos, uno con tierra de un índice bajo de infestación y otro con un índice alto de infestación por la incorporación de gran cantidad de micelio y esclerocios del organismo. En la primera prueba se polvorearon semillas de berenjenas con un desinfectante determinado antes de sembrarse. En la siguiente prueba 10 tiestos fueron tratados con un gramo del producto químico respectivo, para un tratamiento, por cada pie cuadrado de terreno. Cada tratamiento en las pruebas en ambos casos, tratando las semillas y tratando el terreno con fungicidas, comprendían 10 tiestos distribuidos al azar en 10 bloques distintos, bajo techo de cristal. Los resultados aparecen en las tablas 4 y 5, y según el análisis biométrico de los datos obtenidos, se llegó a las siguientes conclusiones:

1. En las pruebas en que se desinfectó la semilla antes de sembrarse, los productos "Semesan," "Z-O," "Dipdust," "Cuprous Oxide," "Corona Coppercarb," "Zinc Oxide," "Semesan Jr.," "Arasan," "Fermate" y "Spergon" demostraron igualmente su efectividad en evitar el salcocho en la fase preemergente de desarrollo de las plantas, pero fueron ineficaces todos en evitarlo después de emerger las plantitas.

2. Los productos "Dipdust," "Cuprous Oxide," "Dupont No. 1," "Zinc Oxide," "Semesan," "Spergon," "2% Ceresan," "1155-H. H.," "Fermate," "Z-O," "Arasan," "New Improved, Ceresan," "Corona Coppercarb," "Cupric Oxide," "Uspulun," "Barbak D.," "Semesan Jr.," "Cuprous Oxide," "Cuprocide" y "Bayer 1494" demostraron ser igualmente efectivos en disminuir el por ciento de infección al compararse con los datos de los testigos.

Las pruebas tienden a demostrar la eficacia de los tratamientos de compuestos de cobre y mercuriales en inhibir o quizás destruir al *Rhizoctonia solani*. Estos fungicidas son efectivos si se incorporan al terreno antes de sembrar la semilla, pero son ineficaces después de aparecer el salcocho. Podemos, por lo tanto, concluir que es una práctica muy recomendable y poco costosa la de desinfectar la semilla y también el terreno del semillero para evitar la aparición de los salcochos. De acuerdo con los trabajos de Nolla (9–11), los salcochos de pimientos, berenjenas y tomates causados por hongos de los géneros *Phytophthora* y *Pythium* pueden evitarse desinfectándose la tierra con vapor *de agua y formalina*, como hemos indicado; con "Corona Coppercarb" a razón de cuatro gramos por pie cuadrado de semillero; y también con dos aplicaciones de caldo bordelés 4-4-50 a razón de medio galón por pie cuadrado de semillero antes de regarse la semilla, seguido de otra aplicación una semana. después de haber germinado la semilla. De acuerdo con los trabajos de este investigador, ningún mercurial probó ser efectivo en el combate de *Phytophthora* y *Pythium*.

En vista de los resultados obtenidos en este trabajo, podemos indicar la conveniencia de usar combinaciones de los tratamientos arriba expuestos y aplicaciones de caldo bordelés para evitar la aparición del salcocho causado por el *Rhizoctonia*, *Pythium* y *Phytophthora*.

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STUDIES ON THE AEREOBIOLOGY OF PUERTO RICO

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The vast and complex conglomerate of living organisms known as the biota of the air is made up of bacteria, fungi and mites. A second important group, the products of living organisms, are insect emanations, pollen grains, and the minute particles of furs, linen, feathers, animal danders, etc. which form legion in house dust. Of the biota the more abundant in the Puerto Rico air are the fungi. The present paper deals with this group.

For the last three years the writer has been studying the fungi of the air with the object of discovering if there is any relation between them and the numerous cases of nasal allergy occuring in the Island. Several facts point toward a close correlation between fungi and nasal allergy. This subject will be dealt with in a forthcoming paper.

All the fungi were obtained by exposing plates of Sabouraud's media for five minutes, in different localities, especially in the residences of asthmatic individuals. Each fungus was then grown separately in test tubes and set aside for determination. The species herein reported are those that were obtained with greater frequency. Slides coated with Brandt's glycerin-jelly were also exposed for twenty four hours, in order to obtain a daily sample of the air content and correlate the study with the quantitative and qualitative data obtained in the cultures.

The number of species of fungi herein reported is 24. Those marked with an asterisk are new to Puerto Rico, while the new species or combinations appear in bold faced type.

I wish to express my appreciation to Dr. Manuel Pila of Ponce, Puerto Rico, whose unaltered inspiration has guided this work and whose unfailing advice has been most useful. Thanks are also due to Miss Marie Betzner Morrow, of the Bacteriology Department, University of Texas, for her help in the determination of some of the fungi here mentioned.

1. CHAETOMIACEAE

1. CHAETOMIUM GLOBOSUM Kunze & Schm.

Perithecia about 300 μ in diameter, olivaceous, clothed with slender hairs; asci clavate.

Isolated from the awnings in the residence of Mr. C. E. Chardón, Mayaguez.

2. PHOMACEAE

2. Coniothyrina ponceana sp. nov.

Pycnidiis globuloso-depressis, membranaceis, sparsis, superficialibus, nigris, contexto fuligineo; myceliis septatis fuscis, sporulis sphaeroides, fuligineis, intense olivaceis, interdum 1-guttatis; chlamydosporiis intercalaribus fuscis.

In solutionibus cultum.   Prope Ponce, Puerto Rico.   Oct. 1945.

Mycelium olivaceous, pellucid, septate, branched, 3 μ thick; ropy hyphae 4–6 μ wide and thick walled, darker in color; pycnidia round, black, without ostiole, membranaceous, 400–600 μ in diameter; chlamydospores intercalary, almost round, thick-walled, immersed in the substratum, in chains, dark-brown, 6–8 μ in diameter; conidia round, olivaceous, with oil drops, smooth, 4–6 μ in diameter.

The genus *Coniothyrina* was established by Sydow (Ann. Myc. **10**: 233, 1912) to replace *Coniothyrella* Speg.; which was untenable because of priority.   As defined it differs from *Coniothyrium* Corda in possessing superficial pycnidia without ostiola.

Although we have placed this fungus under the above genus we do it with some degree of hesitancy.   The growth in Sabouraud's medium is typically penicelloid, the mycelium presents the same type of intercalary chlamydospore we observed in some Penicillia and even the ropy masses of mycelium are sometimes observed.   However, the fruiting bodies are typically phomoid and the spores round or ellipsoid, and colored.

3. MONILIACEAE

3. CEPHALOSPORIUM CURTIPES Sacc.

Colonies floccose, white; hyphae creeping, septate, branched, reverse colorless; conidiophores short, arising as lateral branches of the mycelium, conidial heads round; conidia elongate, elliptical, hyaline, 8–10 x 3–4 μ.

Isolated from room of asthmatic patient.   Ponce.   Oct. 10, 1945.

4. TRICHODERMA KONINGI Oud.

Colonies light green, reverse colorless, vegetative hyphae septate, hyaline, conidia elliptic, 3–4 x 2–3 μ, smooth, hyaline.

Isolated from balcony in dwelling of B. Alzamora, Mayaguez, Sept., 1944.

5. TRICHODERMA LIGNORUM (Tode) Harz.

Colonies hyaline, fruiting areas in white tufts, reverse colorless; conidiophores forming whorls; conidia globose, smooth, 3–4 μ in diameter.

Isolated from dwelling of F. Bonin, Ponce.   Oct. 1945.

6. ASPERGILLUS FLAVUS Link.

Conidial areas yellowish, reverse lighter; conidiophores with pitted walls; conidia pyriform, hyaline to yellow, usually rough, 2 x 6 μ in diameter. Sclerotia white.

Isolated from dwelling of F. Bonin, Ponce. Oct. 1945.

*7. ASPERGILLUS FLAVIPES Bainier and Sartory.

Colonies white at first; then yellowish. "Hulle" cells usually present. Reverse yellow to brown, heads columnar, light colored; conidia smooth.

Isolated from parlor at Alzamora's house. Sept. 1944, Mayaguez.

8. ASPERGILLUS NIGER van Tieghem.

Colonies submerged, yellowish. Reverse uncolored. Conidial heads black.

Isolated from Dr. R. Perea's house. Mayaguez, June 1944.

9. ASPERGILLUS TERREUS Thom.

Colonies cinnamon, spreading, velvety. Reverse yellowish; heads long, up to 500 μ; conidia elliptic, smooth.

Isolated from balcony of B. Alzamora's house. Mayaguez, Sept. 1944.

*10. ASPERGILLUS LUCHUENSIS Inui.

Resembling in general character A. *niger*, but heads lighter in color.

Isolated from balcony of B. Alzamora's house, Mayaguez, Sept. 1944.

*11. ASPERGILLUS OCHRACEUS Wilhelm.

Colonies ochraceous, with little mycelium. Conidiophores pitted with yellow; conidial heads globose, radiate; conidia spinulose, yellow.

Isolated from B. Castell's house. Playa Ponce, June 1944.

*12. ASPERGILLUS TAMARII Kita.

Colonies greenish, reverse pinkish, head columnar, with radiating chains; phialides in two series; conidia pyriform, rough.

From Dr. R. Perea's house, Mayaguez, Sept. 1944.

*13. ASPERGILLUS FUMIGATUS Fres.

Colonies green to dark green; reverse yellowish; conidia dark green in mass, globose.

Isolated from parlor in home of B. Alzamora, Mayaguez, Sept. 1944.

14. PENICILLIUM CHRYSOGENUM Thon.

Colonies green, cottony, spreading; reverse yellow; conidiophores separate, about 300 μ long; conidial heads about 200 μ; conidia elliptical or globose, 3–5 μ, pale green.

Isolated from Maldonado's home, Ponce. Oct. 1945.

15. PENICILLIUM CYCLOPIUM Westling.

Colonies in coremiform masses, loose, spreading, surface blue-green, reverse reddish; conidiophores intertwined; heads in columnar masses, about 350 μ long; fructifications in three rows of metulae; conidia globose, smooth, 2–4 μ.

Isolated from M. Vallecilla house, Ponce. Oct. 1945.

16. PENICILLIUM . . . . . VIRIDICATUM Westling

Colonies velvety, green, reverse colorless, conidiophores about 200 μ long;

heads in loose, radiate masses; fructifications in three stages; conidia smooth, globose, light green, 3–4 µ diameter.

Isolated from B. Alzamora's house, Mayaguez.   July 1944.

*17. PENICILLIUM EXPANSUM (Link) Thom.

Colonies gray green, brown with age, floccose, concentric; reverse brown, conidiophores singly; conidial fructifications in 3 series, 120–180 µ long; phialides crowded; conidia elliptical to globose, 2–4 µ diameter, green.

Isolated from parlor of B. Alzamora's house, Mayaguez, July 1944.

18. PENICILLIUM RUGULOSUM Thom.

Colonies yellowish-green, reverse yellow to orange, conidiophores about 200 µ; conidia elliptical, green, verrucose 3–4 x 2.5 µ diam.

Isolated from parlor of R. Perea's home, Mayaguez, June 1944.

*19. SCOPULARIOPSIS BREVICAULIS Bainier.

Colonies white at first, then yellowish brown; conidiophores short; conidia in chains, pear-shaped, in mass light brown to chocolate, 6–8 x 7–9 µ.

Isolated from parlor of Maldonado's house, Ponce.   Oct. 1945.

20. ACROSTALAGMUS CINNABARINUS Corda.

Colonies round, orange to red; conidiophores terminating in branches bearing conidia; conidia oblong, 5–8 x 3–4 µ; head enveloped by slime.

Isolated from Bauza's parlor, Ponce.   Oct. 1945.

21. TRICHOTHECIUM ROSEUM Link.

Colonies white at first, then pink; conidiophores erect, conidia acrogenous, single; forming a head by the apical growth, cell larger, pear shaped, two celled, hyaline, 19–14 x 8–10 µ.

Isolated from porch at Alzamora's house.   Mayaguez, Sept. 1944.

4. DEMATIACEAE

22. STACHYBOTRYS ALTERNANS Bonorden

Sterile hyaphae black brown; conidiophores erect, unbranched; conidia borne on phialides, elliptical, black, echinulate, 8–12 x 5–8 µ.

Isolated from Maldonado's room.   Ponce, Oct. 1945.

*23. HORMODENDRUM CLADOSPORIOIDES (Fres) Sacc.

Colonies dark olivaceous, round, dense; conidiophores erect, branched, olivaceous at the apex; conidia cylindrical to oval, smooth, olivaceous, continuous or septate.

Isolated from sputum and bed-room of Tuti Alzamora, Mayaguez, June 1944.

24. Curvularia pilae sp. nov.

Hyphis sterilibus tenuibus, effusis, flexuosis nodulosisque, septatis atro-fuscis; conidiophoris erectis rigidis fuscis; conidiis obtuse-fusoideis, 2–3 septatis, fuscis, loculo centrali obscuriore dilatati ibique plerumque abrupte geniculatis, loculis terminalibrus dilutioribus subhyalinis.

Curvularia lunata (Walker) Boedjin valde affine, sed conidiis minoribus diversum.

Eximio medico portorricence Emmanueli Pila, sui libenter diccata.

In solutionibus cultum.   B. Alzamora #4 (*typus*) Prope Mayaguez.

Mycelium septate, richly branched, subhyaline to dark brown; hyphae 3–7 μ wide, sometimes nodulose; conidiophores brown, septate, erect, unbranched, dark brown on the lower part, in the upper, lighter and pellucid, sometimes swollen and with knobs, lower cells 6 μ wide, 8 μ long, tip cells narrower, nearly round, 4 μ in diameter, tips obtuse, 190–280 μ long; conidia borne in a whorl at the tip of the conidiophores, two to three septate, straight or curved, brown, end cells light colored, center cell dark-brown, 15–20 x 6–9 μ, ends obtuse.

The genus *Curvularia* was established by Boedjin (Bull. Jard. Bot. Buitenzorg **13:** 120, 1933) by segregating from *Helminthosporium* Link those species characterized by short, few septate conidia.   In addition to the number of septa, a feature of the genus is the curved or bent character of the conidia, due to the growth of the central cell, which becomes larger and darker than the terminal cells.   This makes the spores dark brown in the center and almost hyaline in the ends.   This same character of the spores is a feature of some species of *Spondylocladium* Martius and *Acrothecium* Preuss.

NOTES ON TRETANORHINUS OF CUBA AND THE ISLE OF PINES

By CHAPMAN GRANT

INTRODUCTION

The first specimens of *Tretanorhinus* I had ever seen were those I described from Grand Cayman. The question was whether they were closer to Cuban or mainland forms. The key of Duméril and Bibron, Vol. 7, p. 349, showed that they were closer to the Cuban form. Dr. Barbour kindly sent me a Cuban specimen which he said was a "good typical example." When this specimen arrived it was seen to differ in color and pattern from the Grand Cayman specimens which I described (Grant, 1940, p. 46) as "differing in pattern and color only" from the Cuban form. While the Cayman paper was in press, Mr. Adrian Vanderhorst arrived from Cuba with a small herpetological collection. He presented me with his only specimen of *Tretanorhinus*, which was indistinguishable from the Cayman form except for color differences. Since Barbour and Ramsden (1919, p. 193) say that "this species is remarkably uniform in colouration ...," I decided that there must be more than one species in Cuba of which they had seen only the one with uniform coloration. I had been planning another collecting trip to the Antilles and this prompted me to visit Cuba. At about this time I heard of Congdon Wood's paper (1939) on this topic. Close reading of his paper made it clear that there was still something to be learned. The trip was cut short by the beginning of the War, but I was nevertheless able to collect the series of 59 specimens which is treated herein. The series was taken on the property of the Central Soledad near Cienfuegos, Santa Clara, Cuba, from January 13–23, 1942.

The material available at present in museums is insufficient to satisfactorily delimit subspecies. Mr. Wood's classification was largely based on color and markings, which might have been sufficient if his series had been large. The establishment of a subspecies based on the color of four specimens is hardly convincing, especially when some of the material in museums was originally preserved or set in formalin. Specimens frequently have insufficient ecological data. For instance, all of my series were taken on the Soledad properties in fresh water streams except one which was taken in a tidal estuary. This one brackish water specimen represents a separate species. The average label would not have differentiated the environment of this from that of the others, nor would the catalogue necessarily have made the distinction.

Probably one or more species and or several subspecies of *Tretanorhinus*

inhabit Cuba and the Isle of Pines. When the problem is carefully worked out, the boundaries will doubtless coincide with ecological factors more than with the present vague geographical divisions.

Possibly some modification in nomenclature should be made in Wood's review of the genus in Cuba and the Isle of Pines. Wood uses preoculars, number of scale rows, light or dark venter, spots or crossbands as diagnostic characters. I endeavor to show that in my series, variation in preoculars and spots and bars are largely sexual manifestations and that the color of the venter is individual.

SYNONYMY

T. insulae-pinorum Barbour, probably a synonym.

Probably *T. insulae-pinorum* Barbour, is a synonym of *T. wagleri* (Jan), both being 21 scale row snakes. Barbour (1916, p. 306) gives the two following contradictory descriptions:

> "*Tretanorhinus insulae-pinorum.* sp.nov. This species differs from the Cuban *T. variabilis* in having regularly 21 instead of 19 rows ... I have examined 3 examples ... The series of 9 Cuban examples ... have 19 rows ... There do not seem to be other differences in squamation and the color is the same so far as one may judge from Mr. Link's material preserved in formalin...."

Barbour reverses this diagnosis (1937, p. 154):

> "This species [*T. variabilis insulae-pinorum*][1] seems to have regularly 19 rows of scales while the Cuban snakes have 21. This is, at first sight, a trivial character but one which is apparently really diagnostic."

Wood (1939, p. 7) lists M.C.Z. No. 12,285, *T. v. insulae-pinorum*, as having 20 scale rows. I would have expected that it was really a 21 row snake since an even number of rows is not normal in this species. Mr. Loveridge kindly checked and reported that it had 19 rows. This individual may be an exception, abnormal, a specimen of another species or the labels may have become mixed. This is discussed later.

In Cuba there are snakes with 19 and 21 scale rows, which might account for Barbour's change of diagnosis, but I believe that the original description giving 21 rows is probably correct for the Isle of Pines freshwater population.

Tretanorhinus variabilis wagleri (Jan)

1865 *Helicops wagleri* Jan, Arch. Zool. Anat. Phys., Vol. 3, p. 247: Icon. Gen., Vol. 28, pl. 1. fig. 1, 1868.

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[1] Barbour reduced his *T. insulae-pinorum* species to subspecific rank not because of any additional evidence of intergradation, but, as he says, merely to designate relationship.

1916   *Tretanorhinus insulae-pinorum* Barbour, Ann. Carnegie Mus., Vol. 10, pp. 306–307; Zoologica, Vol. 11, p. 110, 1930; idem. Vol. 19, p. 134, 1935; and Loveridge, Bull. M.C.Z., Vol. 49, p. 351, 1929.

1937   *Tretanorhinus variabilis insulae pinorum* Barbour, Bull. M.C.Z., Vol. 82, p. 154.—Wood, Proc. New England Zool. Club., Vol. 18, p. 6.

1939   *Tretanorhinus variabilis wagleri* Wood, Proc. New England Zool. Club., Vol. 18, p. 7.

Type.—Unknown to writer.

Diagnosis.—A fresh water form from the Isle of Pines and western Cuba, intergrading at some place or zone with *T. v. variabilis*. Females usually, and possibly constantly, with 21 rows; tail as much as 10 caudals longer than in *variabilis variabilis*; color pattern with possible minor differences.

Tretanorhinus variabilis variabilis Duméril & Bibron

1854   *Tretanorhinus variabilis* Duméril & Bibron, Vol. 7, p. 349, pl. 80, fig. 4.—Cope, Proc. Acad. Phila., p. 298, 1861; idem. p. 309, 1868.—Jan, Arch. Zool. Anat. Phys., Vol. 3, p. 254, 1865.—Gundlach, Erp. Cub., p. 80, 1880.—Bocourt, Le Natur., p. 122, 1891.—Boulenger, Cat. Sn. Brit. Mus., Vol. 1, p. 282, 1893.—Barbour, Mem. M.C.Z., Vol. 44, p. 330, 1914; idem. Vol. 47, pp. 192–194, 1919; Zoologica, Vol. 11, p. 110, 1930; idem. Vol. 19, p. 134, 1935.

1861   *Tretanorhinus cubanus* Gundlach, Mon. Berlin Ac., p. 1001; Erp. Cub., p. 81, 1880.—Bocourt, Miss. Sci. Mex. Rept., p. 795, 1895.

1865   *Tretanorhinus variabilis* var *adnexus* Jan, Arch. Zool. Anat. Phys., Vol. 3, p. 247.—Bocourt, Le Natur., p. 208, 1891.

1883   *Helicops variabilis* Garman, N. Am. Rept., p. 33.

1937   *Tretanorhinus variabilis variabilis* Barbour, Bull. M.C.Z., Vol. 82, p. 154.—Wood, Proc. New England Zool. Club., Vol. 18, p. 9, 1939.

1939   *Tretanorhinus variabilis adnexus* Wood, loc. cit., p. 8.

Type.—Paris Museum.

Diagnosis.—A fresh water form from the eastern part of Cuba; 19 scale rows counted behind neck; subcaudals, male not over 70, female 54; color similar to *T. v. wagleri*; intergrading with *wagleri* at an undetermined place or zone.

Tretanorhinus gaigeae sp. nov.

Type.—Male, No. 60 Grant Cuban Coll., C. Grant coll., in brackish tidal estuary at Rancho Gavilan, Cienfuegos, Cuba; Jan. 18, 1942; adult male.

Diagnosis.—Upper parts light gray; 19 scale rows counted behind neck; small dorsal spots or saddles instead of crossbars as in *variabilis*; a con-

tinuous dark line between ventrals and first row; first, second and part of third rows cream color; a broken black line on upper part of third row; above this, gray. Color and markings not approached by any specimen in a series of 58 specimens of *variabilis*.

Description.—Squamation as in *v. variabilis*; ventrals 154, caudals 68, preoculars 2–2, loreals 1–1; body 581 mm., tail 175 mm.; belly cream colored, finely speckled with brown; 34 dorsal spots neck to sacrum, about 20 on tail.

Wood says of M.C.Z. No. 12, 285: "Nueva Gerona, is gray, with crossbars for the most part broken into small spots, resembling the mainland *nigroluteus* in this respect. Dark lines on first and fourth rows, the latter broken into spots posteriorly. Second and third rows cream colored. Below brown, speckled with white. Upper half of rostral, internasals, prefrontals and temporals light cream colored. Frontal dark brown; parietals light brown, finely speckled with black. This specimen with its extremely odd color pattern and unique scalation is probably no more than a freak."

Both specimens are males and it is possible that the female has 21 scale rows. Dr. Dunn examined my No. 60 and said that he was impressed with the difference in color.

DISCUSSION OF *Tretanorhinus v. variabilis* D. & B.

Habits

At the time of my visit to Cuba, Dec. 13–23, 1941, it was very dry. Many of the smaller streams were a succession of puddles swarming with small fish which were being preyed upon by birds, crabs and snakes. The snakes were in turn being mutilated and even killed and eaten by the crabs. Many snakes were taken which had old or fresh scars on various parts of the body. The snakes come out of hiding about an hour after dark and start hunting fish. Snakes taken as late as 10 P.M. contained little food, but those found in the mornings resting on the mud under rocks or debris were full of fish. The snakes are quiet, easily caught and make no attempt to bite, but once frightened they show great agility in hiding. When taken by hand one characteristic is at once noticed at variance with the habits of most snakes: they grasp one's wrist with their tails with considerable force. A specimen can hold up the weight of its body if its tail is allowed to grasp one's finger. The nocturnal habits of this snake are attested by the vertically elongated pupil. There were no enlarged ova, but the knobbed scales on head, neck and region of the vent of males were prominent, a modification which is apparently permanent after maturity.

Barbour (1914, p. 330) says "It is a strictly aquatic snake which never leaves the water. . . . It is a difficult species to find." (1916, p. 306) "The catibo leads a colorless existence . . . The members of this genus are the

most strictly aquatic reptiles I know, quite equalling the Hydrophids in this respect. I have never heard of their eggs being found, and I have often wished I knew whether they come ashore to lay. I presume that they do."

Scale Rows

Serpents have probably undergone a general reduction in the number of body rows through modification of the body itself while evolving from a lizard-like form. The head of the snake is therefore the most logical place to look for remnants of lost rows since it has changed less than the body. Every neck scale or scale bordering the head-plates may have some significance although not always possible of interpretation. On the other hand there are cases among serpents where the number of body rows has increased with an increase of body size. This reversal of the general evolutionary trend is to meet some obscure mechanical law correlating the size of the scale and consequently the number of rows to the size of the snake. The correlation varies in different genera also due to obscure reasons connected with ecological factors and physical characteristics or manner of locomotion of the genus. *Typhlops* reduces by dropping a ventral row next the center; an indication of the order in which other serpents have lost their midventral suture. In the case of divided anals or preanals in *Tretanorhinus* the preceding ventral projects an angle posteriorly in an attempt to cover the suture. When a snake is found to have more rows at midbody than at the anterior part of the body it is probable that a short neck row occupies the same relative position as the added row.

Dr. Dunn points out that the hooded cobra's numerous neck rows may be a result of the hood and not representative of primitive body rows.

I have elsewhere (Grant, 1937) discussed the probable non-existence of a "midventral" row in reptiles except in snakes. The middorsal row may have had a different origin from the body rows. It may have originated with callosities, scutes or spines covering the vertebral processes as is now seen in the crests of lizards. It possibly did not develop from a fusion of the two highest body rows. The spiny crests of lizards are usually interrupted or reduced at neck and sacrum as is the spinal or vertebral row in snakes.

Ruthven (1908, pp. 16–21) found that the first rows to be dropped in *Thamnophis* were about midway between the ventrals and the spinal and were dropped in a fixed order somewhere near the middle of the body. Blanchard (1921, p. 10) stated that *Natrix* did the same.

Tretanorphinus, however, drops the paravertebrals at about the 100th ventral plus or minus ten, but apparently without dropping the fourth or fifth rows.

I have written on the origin of the rows on the head of *Natrix* (Grant, 1935, p. 927) pointing out that frequently two rows start at the juncture of the parietals and extend only a short distance when the spinal or vertebral row appears. The spinal row does not appear to be a fusion product of the two dropped rows (see fig. 1.). Thus the paravertebrals are dropped by all three genera, but at widely different places.

Natrix and *Thamnophis* drop rows 4, 5 and 6 in a fixed order at about midbody. There is no such reduction apparent in *Tretanorhinus* until the neck is examined when it is seen that there is a short fourth row which
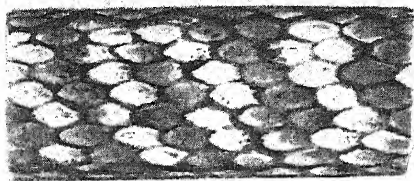
Fig. 1. Squamation of head of *Natrix sipedon* showing origin of scale rows.

stops at about the twelfth ventral. Thus the three genera seem to drop the same rows, but at widely different places.

To recapitulate: *Natrix* shows rudimentary paravertebrals and drops fourth etc. at about midbody. The "19 row" *Tretanorhinus* drop the paravertebrals at about midbody and the fourth row is rudimentary. Therefore one might expect to find that the "21 row" *Tretanorhinus* extended the 4th row to about midbody.

The very first 21-row snake examined from the U. S. N. M., No. 27,980 carried out this expectation. The 4R drops at 72 and the 4 L at 18. This seemed to show a direct similarity between the genera in this respect, ex-

cept for the fact that all the other U. S. N. M. 21-row specimens examined did no such thing; they drop the 10th and 9th rows.

This brings up the possibility that larger series may prove that there are two species; one dropping the 10th and 9th and having a rudimentary 4th and another species which drops the 10th at midbody and the 4th also at midbody.

My Soledad series are all 19-row snakes as commonly understood. However, all can be termed 21-row snakes if the rudimentary 4th row which extends to about the 12th ventral be counted. They then could be termed 21-19-17 row snakes, but by present usage they are all 19-17 row snakes since a rudimentary or midbody row is seldom noted. This fact shows the advisability of giving the first count as the maximum midbody count and so stating or explaining definitely any other system used.

Dr. Dunn has this to say: "It is a general characteristic of Xenodontine snakes to reduce from midbody by dropping paravertebrals; of Natricine and Colubrine snakes to reduce from midbody maximum by dropping laterals. There is lots of evidence as to this in many genera and species. What happens on the neck is as yet not widely known, nor for many forms. It would be interesting to know more and the odd U. S. N. M. 27,980 is interesting."

Scale rows of the U. S. N. M. specimens

Disregarding the short neck rows it will be remembered that the Soledad series dropped the 9th row at 100 plus or minus ten. The 6 U. S. N. M. 19-row specimens drop the 9th or paravertebral row at from the 90th to the 123rd, averaging 111. The six 21-row specimens present an entirely different picture. Instead of having an extended 4th row as one might expect, the 10th and 9th rows are dropped, the 10th at from the 52th to the 87th, averaging 71; the 9th at from the 116th to the 142nd, averaging 130. Two of this latter six were irregular. No. 27,499 has irregular rows that appear and disappear in a most confusing way. In places this specimen can be counted as a 23 row snake. I have seen such examples in *Natrix*.

There may be two species of 21-row snakes; one with extended fourth row which drops at about midbody and the 10th somewhat farther back; another species which drops the 10th and 9th. If these two forms do exist the female of each may be a 21-row and the male a 19-row form.

Errors possible in counting rows

The spinal row starts several scales behind the parietals and disappears a few scales anterior to opposite the vent. A count just anterior to the vent might result in an even number which would not be a true count. Occasionally there is a short row near the vent between rows 2 and 3 or 3 and 4, which, if counted would give a count of two too many. Frequently

a row is dropped as much as 10 scales anterior or posterior to its mate on the opposite side. This usually accounts for the even-number counts frequently seen. The writer remembers one *Natrix* in which a row on one side was represented only by two small scales and a third which appeared at a short distance.

An example is U. S. N. M. 27,980. Wood lists this snake as having 20 rows. This snake drops the 4R at 72, the 4L at 18; 10 R & L at about 129. Thus it has 21 rows from 1 to 18; 20 rows 18 to 72; 19 rows 72 to 129; 17 to vent. Loveridge calls it a 21-row snake; Dunn (letter Nov. 24, 1944) and Dr. L. M. Klauber call it a 19-row snake with an abnormally long 4R.

Since the "usual counting place" is half way between snout and vent, when a species of snake having a spinal row counts out an even number, the cause should be discovered and the explanation given. An even number is probably caused by an abnormality.

DATA ON SOLEDAD AND U. S. N. M. SPECIMENS

Size and proportions

The females appear to be larger judging by this series and the Cayman Island species. The tails are proportionately shorter than the males and the young have tails proportionately shorter than the adults.

Secondary sexual differences

Males are smaller, have shorter bodies with fewer ventrals; longer tails with more caudals; predominate with a single loreal; have greater proportion of preanal sutures; have heavier keels and striations and when mature have knobs on chin scales and near vent. The female has a greater proportion of 3 preoculars and has more acute abnormalities than the male.

In all specimens recorded by Wood every one with 21 rows or with preoculars 3-3 is a female, but he does not stress this fact. The only specimen Wood lists with 21 rows that he does not list as a female is his p.8; "U. S. N. M., No. ?, 21 rows, sex ?" Miss Cochran kindly furnished the number as 27,499 and the sex, female.

The anal and preanal

The anal and frequently the preanal is divided by a diagonal suture running forward from the snake's right to left. The direction of this diagonal in this species is invariable (Grant, 1944). There is occasionally a half ventral entering from either side immediately anterior to the anal or preanal or between the two.

The anal region is modified in 58% of males and only 16% of females. This discrepancy seems to have a direct sexual significance and might be considered in the category of secondary sexual dimorphism. The 12 U. S.

N. M. specimens examined had no divided preanals, but only two were males.

A divided anal, preanal or a half ventral in this region is preceded by a pointed ventral, the point tending to protect the suture. This may be an indication of how important for survival it was for snakes to develop single ventrals.

Males with split preanals seem otherwise normal, but one of the two females thus split seemed to be generally abnormal, having abnormal loreals and temporals and she was the only specimen in the whole series with three postoculars in addition to having an extra half ventral.

Certain scales nearly free from abnormalities

The frontal, prefrontals, internasals, parietals, rostral, mental and chin shields show practically no abnormalities. In two cases the corner of the prefrontal enters the orbit and this might be considered as a fusion of that scale with the preocular. The posterior angle of the frontal is subject to considerable variation in outline. The supraoculars occasionally seem to fail to pinch off the uppermost preoculars on one side.

Scale abnormalities

Head abnormalities are more numerous in females. Omitting the labials there are 26% male and 60% female abnormalities; including labials there are 60% male and 180% female abnormalities. The term "abnormality" used here could be supplanted by "unusual" or in some cases "supernumerary".

Individual variations in tabular form

	34 males	25 females
Loreals....	1–1, 1–2, 2–2	1–1, 1–2, 2–2, 2–3
times oc-curring..	23 4 7	10 5 8 2
Preoculars	1–1, 1–2, 2–2, 2–3, 3–3, 4–4	2–2, 2–3, 3–3, 4–4
times oc-curring..	1 1 8 11 12 1	4 1 19 1
Ventrals...	150, 1, 2, 3, 4, 5, 6, 7, 8, 9, 160, 1, 2, 3 1 1 1 2 9 5 3 4 4 3 0 0 0 1	156, 7, 8, 9, 160, 1, 2, **3**, **4** 2 0 6 3 4 4 1 2 3
Caudals...	2 broken not counted 60, 1, 2, 3, 4, 5, 6, 7, 8, 9 1 6 8 5 4 2 3 0 2 1 average 63.5; range 9	4 broken not counted 48, 9, 50, 1, 2, 3 1 4 7 4 1 4 av. 50.7; range 5

Note actual gap of seven counts between sexes in caudals and that females have more ventrals and fewer cuadals.

Labial variation

Small triangular scales are wedged between the lip-corners of certain labials. Their shape and position give no clue that they were derived from either of the adjacent labials. Normally there are 8 upper and 10 lower labials. Of 34 males 9 had 11 irregularities; of 25 females 13 had 32; 26% males and 52% females had irregularities; irregular males averaged 1.22 irregularities; females, 2.46. Nearly 50% of irregularities were between the 4th and 5th lower labials.
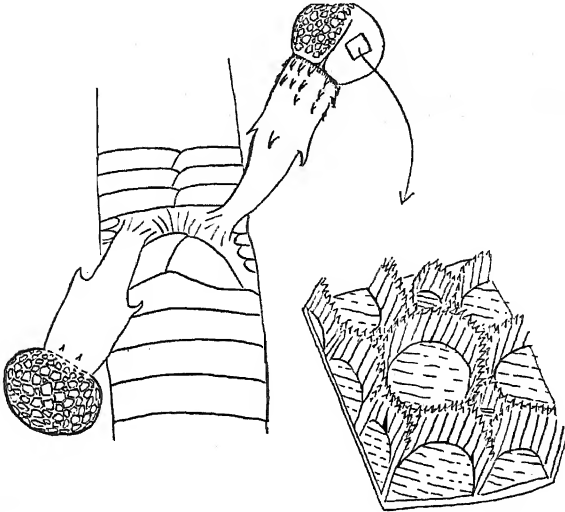
FIG. 2   FIG. 3

FIG. 2. Hemipenes of freshly killed *Tretanorhinus v. variabilis*, injected.
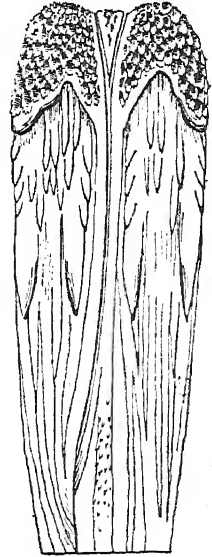
FIG. 3. Hemipenes of *Tretanorhinus v. variabilis* drawn from a dissection, after Cope, Report of U.S.N.M., 1898, Plate 23, fig. 11.

Hemipenis

The drawing of the hemipenis herewith was made from specimens in which the organ was injected with alcohol immediately after death. This engorges the organ to such an extent as to hide the sulcus. (Fig. 2). Cope's fig. 11 of plate 23, shown as Fig. 3, was probably made from a dissection. Each figure shows characters not shown in the other.

Color pattern and variation

The venter ranges from cream through various degrees of pigmentation to almost solid slate in different individuals. The dark color displaces the light by dendritic patterns. There is a dark or nearly black line at the

juncture of ventrals and first row.   This line may not be apparent when the
venter is dark.   A light band occupies the remainder of the first, all of the
second and part of the third rows.   This band may be greatly reduced by
pigmentation or may be chocolate.   From the upper part of the third row
the true dorsal pattern begins.   Fundamentally it consists of about thirty
cross bars nape to vent; twelve on tail.   The bars occupy one to three
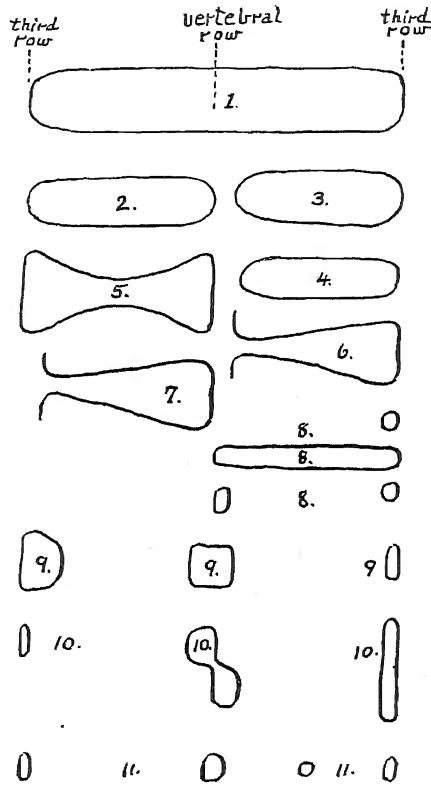
FIG. 4. Diagrammatic drawing of the various color patterns found on *Tretano-
rhinus v. variabilis.*

scales in width, separated by three to five scales; bars are occasionally out-
lined by light and there may be false shadow-bars between them.   No
specimen has been seen that adheres to any one dorsal pattern for its entire
length.   The most common variation is for the pattern on the two sides
to alternate.   Considering the bars on one side: they may narrow at the
middle and widen at the ends becoming hour-glass shaped; they may sepa-
rate, the upper part remaining a spot or coalescing with its mate on the

opposite side forming a saddle; the lower part may remain a spot, but it usually forms a dash of various lengths and in one case a continuous wide stripe; the spots and bars may alternate; the spinal may be clear or have an occasional fleck or it may be black and the bars may touch it or not; the pattern may be reduced to occasional flecks on spinal, mid-side and third row. There may be combinations of these patterns on various parts of the body, but the predominately spotted patterns are confined to the males.

It is not a simple matter to count the bars from neck to sacrum. It is doubtful whether two students would obtain the same counts in a series of specimens.

As is to be expected, the pattern is more clearly defined on young specimens, but does not change in any way with growth. It does become obscured to varying degrees by added pigment. Another change that comes with maturity in many cases is a chocolate pigment which appears on the first three rows or the lateral stripe. The background is rarely chocolate.

Patterns expressed in their simplest terms, by sexes, taken at the vicinity of the 100th ventral:

Pattern	male	female
1. simple bars	8	8
2, 3, 4. bars, spinal clear	0	1
5. hour-glass	13	12
6. bars, spinal black	2	1
7. bars, third row black	1	0
8. bars and spots alternating	1	3
9. saddle and dash or spot	2	0
10. spot and dash	6	0
11. dots only	1	0

Dr. Oliver kindly read this MS and challenged this sentence by saying; "Isn't obscurity a change?" Yes, obscurity is a change in degree of pigmentation, but my meaning is a change in pattern such as spots growing into bars etc. during growth of the individual.

As to patterns I believe that possibly the primordeal coloration of many animals was a plain dark dorsum grading into a lighter venter. This is the simplest pattern and could be considered to consist of two longitudinal stripes—an upper and a lower color. This pattern gradually became more complicated and in turn the more numerous stripes broke up into checkered patterns and these in turn coalesced into cross bars by the process of natural selection. I do not believe that a pattern ever migrates on an individual. Slow moving snakes and animals and/or the young of fast moving adults may be spotted or barred. In the first instance the bars are permanent; in the second they are retained while useful. A pattern

that fades out is usually replaced by a solid color, but in the case of *Sphaerodactylus cinereus* Wagler, the cross bands of the young are replaced by fine dots on a gray ground.   The design has not migrated; it faded out and was followed by another.   In some sexually mature male *Pseudemys* sp. we find a migration of dark pigment towards the sutures leaving the centers of the scutes light.   Again it is not a pattern migration since the true pattern of ocelli, when present, does not move or change other than to fade.   Neither do we consider this last case to be melanism as Barbour chooses to call it; melanism being congenital.

Dr. Dunn states in letter 11/24/44; "The general opinion is that spotted pattern precedes striped or ringed or uniform color in development and in phylogeny (cf. Zenneck, Zeichnung der Boiden). I do not wholly subscribe to the general opinion, but I have seen spotted young change into uniform adults and spotted young change into striped adults, and spots in the embryo which are stripes at hatching.   It certainly happens in development.   I refer specifically to *Elaphe quadrivittata, Lampropeltis rhombomaculata* and *Leimadophis bipraeocularis* (whose 11 month development in the egg I followed in Bogota).   The question is whether development implies anything as to phylogeny as general opinion says it does?"

Dunn does not make it clear whether he refers to the disappearance of a pattern which is followed by another, or whether there was a true migration of a pattern or part of a pattern.

The color patterns of my series may be expressed sexually: males have about 70% barred and 30% spotted pattern whereas the females are 100% barred.   The male venters are 26, 53 and 21% light, medium and dark respectively whereas the females have 28, 64 and 8%.   There is a natural correlation between spotted pattern and light venter; the spotted pattern requiring less pigment than the barred patterns.

Migration of pattern with growth was considered by Barbour who says (1919, p. 193): "In the young also the dark cross bands appear as oblong or squarish dorsal patches which become extended into strap-like bands with increasing age."   I have four very young specimens which bear the entire strap-like bands and not the squarish dorsal patches, and adults which bear the squarish dorsal patches.   I know of no snakes in which the markings spread with growth.   In this and many other species the pattern tends to become dim or almost obliterated by an increase of dark pigment, but the basic pattern remains unchanged.   In the common blue racer, *Coluber* sp., the young have a pattern of cross bars which fade out as the snake grows, but the pattern does not change shape or move.

On the same page Barbour quotes Boulenger as saying: " . . . a more or less distinct light lateral streak on the second and third rows of scales . . ." to which Barbour replies: "The white lateral band, he mentioned is always found in the young, but we have never observed it on adults."

In three of my young specimens the band is distinct and in the fourth dim. Among adults it is occasionally distinct, but usually dull or obscured.

Dr. E. R. Dunn on *Tretanorhinus*

Dunn 1939, writing on the mainland forms says; "The Antillean forms are the subject of a separate report by Mr. G. Congdon Wood, but I have examined the material with him and each of us has had access to the findings of the other.

"For the genus as a whole three groups can be made out. These differ in range, in markings and in ventral count as shown below:

	Atlantic drainage, mainland	Antillean	Pacific drainage, mainland
Dorsal markings	two rows of dots	crossbars	three dark stripes
Male ventrals	133–139	152–162	166–169
Female ventrals	138–151	154–168	168–177."

My Soledad series agrees with Dunn's ventral counts being, males, 150–163, females 156–164.

Congdon Wood on *Tretanorhinus*

Some of the data in Wood's paper seemed out of line with the Soledad specimens. Dr. Barbour kindly forwarded me under date of June 9, 1942, data which Mr. Loveridge had been kind enough to look up. Dr. Cochran kindly loaned me 12 specimens which seemed to have inexplicable data. Mr. Charles Shaw, who has done much counting and sexing for Dr. Klauber, kindly sexed the U. S. N. M. specimens, as I wanted corroboration in making any corrections in Wood's paper.

Some of Wood's data which seemed unconvincing were apparently wrong as shown below. Three cases of sexing which he omitted are supplied.

Specimens		Wood's data	Loveridge's data
M.C.Z.	12,285	20 rows	19 rows
	7,932	female	male
			sexes by Shaw
U.S.N.M.	27,980	sex ?, rows 20	female, 21 rows
	59,322	sex ?, V.?, C.?	female, V. 161, C. 70
	56,367	loreals 1	loreals 3–1 or 2–1 ....
	56,362	male	female
	83,318	C. 67 plus	C. 57 plus
	27,499	sex ?, V.?. loreals 1	female, V. 160, lor. 2–1.

Wood uses trinomials in his synonymy in quoting original descriptions where a binomial was used by the author.

Some of Wood's data which seemed out of line are correct. His specimens seem to have about ten more caudals than the Soledad series; spotted females; fewer abnormal scutes; more scale rows.

The characters which Wood uses as of subspecific value are; (1) color of venter, light or dark; (2) 2 or 3 preoculars; (3) spots or crossbars; (4) 19 or 21 scale rows.

The Soledad series shows the light or dark belly to be purely an individual variation and I believe that it is probably so in other localities. The matter of 2 or 3 preoculars seems to have a sexual significance in all specimens listed by Wood as well as the Soledad series. I attribute the predominance of 3 preoculars in females to the fact that they are the larger sex and heavier headed. The Soledad series seemed to show that spots were strictly a male pattern, but the U. S. N. M. specimens seen by me seem to disprove this assumption. Larger series are needed to settle this point. 19 or 21 scale rows is apparently diagnostic and it would be of interest to know whether a large series would still show the 21 rows confined to females. Data showing where 20 and 19 row populations intergrade would be of value in defining subspecies.

Every specimen in Wood's paper with 21 rows is a female; every specimen with preoculars 3-3 is a female.

Dr. Dunn Nov. 24, 1944 writes; "Wood described no new forms, but made a tentative allocation of a number of existing names. Your big Soledad series has pretty much eliminated his ventral color characters and his preocular characters. There is left the occurrence of 21 scale rows in the west—nearest to 21-row *nigroluteus* of the mainland—and perhaps more spotting and less crossbarring in the west—nearest to the never-barred *nigroluteus* of the mainland. He helped open the situation for discussion by showing that 21 row snakes were not confined to the Isle of Pines, but also occured in Pinar del Rio."

SUMMARY

Little is known about *Tretanorhinus* from Cuba. Individual variation is so considerable that a large series and extensive collecting will be necessary before any definite picture can be formed.

It appears that the number of caudals, pattern, color, preoculars, number of scale rows and possibly the sequence in which the rows are dropped have either taxonomic or sexual significance or both.

There probably is a salt or brackish water form common to Cuba, the Isle of Pines and surrounding islets. This form is probably at present represented in museum collections, but unidentified. The fresh water form has doubtless speciated considerably. The genus seems to be very plastic.

From our present knowledge the following forms appear to be recognizable:

T. v. wagleri (Jan): fresh water of the Isle of Pines and western Cuba; a long tailed form with 21 scale row females; ten more caudals than *T. v. v.*

T. v. variabilis D. & B.: fresh water from eastern Cuba; 19 rows; caudals male 70, female 54.

T. gaigeae, sp nov.: a light gray spotted form, probably confined to brackish water.

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A NEW NAME FOR *ALSOPHIS ANTILLENSIS*

By CHAPMAN GRANT

Schlegel (1837) described *Psammophis antillensis* from St. Thomas, Guadeloupe, Martinique and Cuba. Brongersma (1937) revised this composite species, but left a few loose ends which this paper endeavors to catch up.

The *Alsophis* which inhabits most of the islands and islets east of Puerto Rico has long been known as *Alsophis antillensis* (Schlegel). If a sufficiently large series of specimens could be assembled from each islet of this area, an average difference, possibly of subspecific value, between many of the populations would be apparent. The large series which I have collected from some of the islands tends to bear this out (Grant, 1932). On the other hand, many specimens from most of these localities could be matched in a large series from almost any other island in his area.

Color pattern has played an important part in the taxonomy so it may be advisable to explain how Stejneger's fig. 174 came to be considered typical of the pattern of *antillensis*.

Stejneger habitually described a single specimen of a species in detail and then discussed variations in a separate paragraph. When he summarized *Alsophis antillensis* (Stejneger, 1904; p. 704 et seq.) he had material from St. Thomas, which was one of the type localities, but these specimens were not in good condition. Therefore he selected a half-grown, distinctly patterned specimen from Culebra Island, USMN No. 25557, and illustrated its color pattern at midbody with his Fig. 174 (see cut). He did not state that this pattern was typical of the species. He merely remarked that the marking on the fifth scale row appeared to be constant, but did not state how far it extended posteriorly, nor did he mention the pattern depicted on the eighth row in Fig. 174. He said: "On the whole the coloration is much as in the specimen described above. . . ." The specimen "described above" was not the one figured, but No. 25554, an adult, doubtless with a dimmer pattern than that of the half-grown specimen figured.

I have found in well-preserved material from the Virgin Islands area that the pattern on the eighth row is usually wanting and the pattern on the fifth may not extend beyond the region of the neck. Anteriorly the pattern on the fifth row may be duplicated on the sixth row and an inverted pattern may be found for a short distance on the fourth row. Note that the fourth row is dropped at about the 108th ventral, or posterior to the middle of the body. If the pattern extends beyond this point it appears to occupy the fourth row, but it is in reality on the same row on which it originated

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the fifth. In my observation a one row pattern does not switch from one row to another.

Stejneger (p. 704) pointed out that the type localities of *Alsophis antillensis*, having been designated St. Thomas, Guadeloupe, Martinique and Cuba, obviously constituted a composite species. Schmidt (p. 140) agreed, but added that "the name has come to be restricted to the Virgin Islands form by the consensus of opinion among herpetologists." The specimen that Schmidt summarizes is not from the Virgin Islands despite his restrictions, but was taken on Culebra. He even records the species from Puerto Rico (pp. 139, 141). He states (p. 139) that Günther (1859; p. 210) restricted the species to St. Thomas, but Brongersma (p. 3) denies this.

Schmidt requested Brongersma, who had access to the type material, to make an examination and publish his findings, which he did. The results of Brongersma's paper may be thus summarized.

SUMMARY OF BRONGERSMA'S PAPER

1. *Psammophis antillensis* Schlegel, is a composite species which the describer thought had wide distribution.

2. A lectotype must be selected to restrict the name *antillensis* to one of the components.

3. The lectotype must be selected from among the specimens upon which the description was actually based and not on others which Schlegel merely examined.

4. Schlegel mentions three cotypes of which the measurements are the only clues to their identity. These specimens are in the Leiden Museum:

a) No. 767 Leiden, labeled *Psammophis antillensis*, from Martinique, is in reality *Eudryas boddaertii* (Sentzen), from Venezuela.

b) No. 768 Leiden, labeled *Psammophis antillensis*, from Guadeloupe, chosen by Brongersma as the lectotype, is identical with *Alsophis leucomales leucomales* (Dum., Bibr. & Dum.), from Guadeloupe.

c) No. 769 Leiden, labeled *Psammophis antillensis*, from St. Thomas, collected by Richard, is in reality *Alsophis sancticrucis* (Cope), from St. Croix.

5. Schlegel did not use St. Thomas specimens in describing *Psammophis antillensis*.

6. As a result of Brongersma's action the Guadeloupe form becomes *Alsophis antillensis* (Schlegel) and *leucomales* becomes a synonym.

7. The St. Thomas form is unnamed unless, as Schmidt says, (pp. 139, 141) it is identical with *Alsophis anegadae* Barbour, in which case it takes that name.

DISCUSSION

I will endeavor to show that:

1. Leiden No. 769, labeled St. Thomas, collected by Richard, is not *A. sancticrucis*. That neither Brongersma nor Schmidt proved that it came from elsewhere than St. Thomas as labeled.

2. Schmidt did not prove *A. anegadae* identical to the St. Thomas area populations and I endeavor to show that it is different.

3. *A. nicholsi* Grant from Buck (or Capella) Islands is subspecifically different from the St. Thomas area populations and becomes *A. nicholsi nicholsi* Grant.

4. St. Thomas etc. have a population differing subspecifically from *A. nicholsi* and is therefore given a new subspecific designation.

Brongersma (p. 3) eliminated Leiden No. 769, labeled *Psammophis antillensis*, from St. Thomas, as a possible lectotype on the following grounds; "The coloration of the anterior part of the body is not that which Stejneger (p. 705, fig. 174) and Schmidt (p. 142, fig. 47) describe as typical for *Alsophis antillensis* from St. Thomas." Note that Brongersma infers that the descriptions and figure referred to are of a St. Thomas specimen. Reference to Stejneger shows that his fig. 174 is of USNM No. 25557 which was taken on Culebra Island and not on St. Thomas. Stejneger's description (pp. 704–705) is of USNM No. 25554 also from Culebra. Schmidt's fig. 41 is a copy of Stejneger's fig. 174. Schmidt (p. 141) says: "Much the best description extant is that of Stejneger, based on a Culebra specimen. ..." Apparently the only color pattern of which Brongersma was aware was that of Culebra specimens.

Probably Brongersma would have avoided a change from a long established name by selecting Richard's St. Thomas specimen for the lectotype had he known all the facts. As it was he sent a "rough sketch" (p. 3) (see cut) of Richard's St. Thomas cotype, 769 Leiden, to Schmidt who identified the sketch as *Alsophis sancticrucis* (Cope) although Schmidt had neither St. Thomas nor St. Croix specimens for comparison. However, the sketch showed what appeared to be crossbands and Brongersma (p. 3) says the specimen has more or less distinct crossbands. On p. 4 he states that there is a specimen in the Paris Museum, No. 3574, also labeled "St. Thomas, Richard," I believe that since this specimen is unchallenged as a St. Thomas specimen, Richard's labels have not been proven untrustworthy.

I thought it necessary to locate a specimen of *sancticrucis* so that the above mentioned sketch might be compared to a real specimen. Mrs. Gaige of the MZUM, Mr. Loveridge of the MCZ, Mr. Bogert of the AMNH and Miss Margaret Storey of Stanford University all kindly answered my query to the effect that they had no specimen of *sancticrucis*. Dr. Cochran obligingly stated that the USNM had a specimen catalogued as *sancticrucis*

USNM No. 11105 from Guadeloupe, but the locality makes this specimen without interest in this case.   Dr. E. R. Dunn kindly reported that the ANS had the type, No. 5404 from St. Croix.   I therefore forwarded him the sketch for comparison.   He stated in a letter dated Feb. 1, 1945:

"I checked the sketch of Leyden 769 directly with ANS 5404, which is one of Cope's original specimens of *sancticrucis*.   The markings of the two differ considerably.   The type has a light line along the meeting edges of scale rows two and three; this does not appear in the sketch.   The type shows light bars running directly across the back; the sketch shows some vague and oblique light marks which do not cross the back.   The ANS has two specimens from the 'West Indies' which agree closely with the type, but are more vividly marked.   In my opinion the Leyden sketch does not represent a specimen of *sancticrucis*."
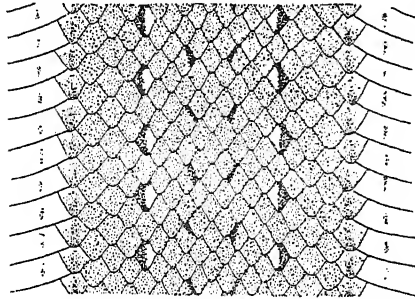
FIG. 174.—ALSOPHIS ANTILLENSIS.   Color pattern around middle of body.   No. 25557, U.S.N.M.

FIG. 5

The reader may compare the sketch, fig. 5 with the photo of the type, fig. 6, for himself.   I invite attention to the "pattern" on the sketch.   A preserved specimen of this genus is prone to lose scales by handling.   When a scale rubs off it leaves a light colored area caused by exposing the lighter colored skin.   The sketch shows what might well be a specimen that had received considerable handling.

I believe that the above evidence, with the illustrations, is sufficient to prove that Leyden 769 labeled St. Thomas is not *sancticrucis* and that there appears to be no reason to doubt that it came from St. Thomas.

There is little doubt but that Brongersma would have given the St. Thomas population a name had not Schmidt (p. 139) synonymized *antillensis* and *anegadae*, although he had neither St. Thomas nor Anegada material at his disposal.   Schmidt stated in a letter to me dated Jan. 11, 1943: "As for the problem of *anegadae*, my reference of it to *antillensis* was based on very general resemblances."   Schmidt (p. 139) includes *anegadae* in the synonymy of *antillensis*.   He explains his action thus (p. 141): "The

two specimens (of *antillensis* from Puerto Rico) agree closely in coloration with the color variety described by Barbour from Anegada and, as I do not wish to admit of a discontinuous distribution of *anegadae*, it seems best to include both Puerto Rican and Anegadian specimens with *antillensis*."
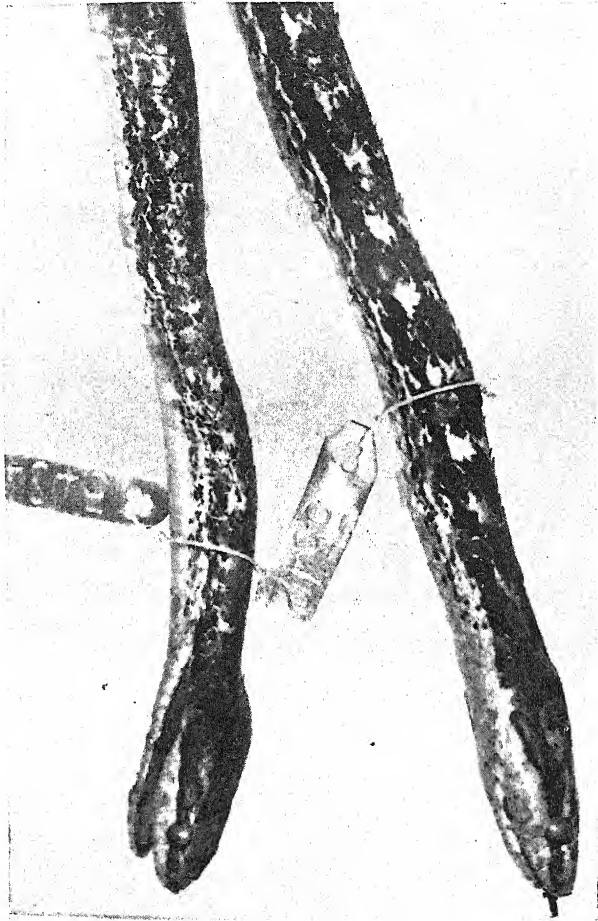
FIG. 6

Brongersma stated (p. 5): "If Schmidt (pp. 139, 141) is right in referring *Alsophis anegadae* to the synonymy of the species occurring in St. Thomas, this name must replace *Alsophis antillensis* auct. (non Schlegel). As I did not examine ... *anegadae* ... I cannot form an opinion on the possible identity ... and ... must leave it to future authors. . . ."

Barbour (p. 102) in his original description of *anegadae* says: "Two

snakes from Anegada are both alike in having a squamation similar to *antillensis* but (differ from *antillensis*) in being pale ashy gray in color, the fifth scale row not parti-colored, but with a median streak of black. The upper lips are immaculate white, unspotted."

I took two specimens on Anegada, both were light tan, not gray, although I have taken gray specimens of this genus on some other islands, Peter Island for example. Both Anegada specimens had the usual parti-colored fifth row scales, differing from Barbour's description of *anegadae*. One, MZUM No. 80639A, has lips almost immaculate; No. 80639B has spotted lips. Both have numerous dark spots on all dorsal rows, a feature occasionally found on specimens from the other islets and are somewhat similar to *variegatus*. I would suggest the following diagnosis for *anegadae*: "A small, pale form differing from the populations on nearby islands in having scale pores much reduced in numbers and having dark flecks on the dorsal scales above the fifth row."

Dr. Cochran kindly sent me 9 specimens of "*A. antillensis*" labeled from St. Thomas and one specimen from Water Island. These specimens divide into three groups as far as pattern is concerned. USNM Nos. 98966, A12403 and 66523 have a dark, broken line (almost continuous on 98966) on the fifth row for the entire length of the body and in addition all rows below the patterned row are dark; rows 7–8 have spots near neck. (Note. Frequently the markings occur between rows or rather occupy the lower part of one row and the upper part of the adjacent row. I designate the rows by number to express this type of marking.) This group presents a pattern different from any I have seen. If it were possible to correlate them with an area, they would represent a well differentiated population. Nos. 66524, 12403 and 75866 have the pattern for half the length of the body and a few spots on 7 near neck; in Nos. 66525 and 66522 the pattern is reduced to about one fourth the length of body. This group agrees pretty well with the "typical *antillensis* pattern". No. 13857 is a snake of the general appearance of the *nicholsi* pattern, described below, with 4–5 marked half the length of the body and 7–8 marked on the neck. These marks are faint and the general appearance of the snake is like *nicholsi*. No. 52547 labeled from Water Island has the appearance of a reduced "*antillensis* pattern" with 5–6 spotted a fourth the length of body and 7–8 marked on neck.

Assuming that all labels are correct it would appear that the St. Thomas population had a greater diversity of patterns than that of any other island in this area and that the Buck or Capella Islands pattern was approached on St. Thomas on USNM No. 13857, reducing the *nicholsi* population to subspecific rank. No "*antillensis* pattern" has yet been taken on Buck or Capella Islands.

My original diagnosis of *nicholsi* states: "A pale form with the squamation of *antillensis* but the pattern of *portoricensis*, namely differing from typical *antillensis* in that the lateral stripe on scale rows four and five is visible only on the neck, where it is very faintly indicated, the broad dark dorsal band is likewise faint and is evidenced only by a gradual darkening of the more dorsal scales and the pattern on the eighth row is missing." Under the description of the type I said: "In life the dorsal ground color is pale olive green, which fades to pale brown in alcohol. This color is light laterally, but becomes more intense dorsally. Each scale with a diffused darker margin. On the neck there is evidence of the characteristic dark lateral band on scale rows four and five, but the characteristic marks which occur on the eighth row of *antillensis* are missing." This quotation shows that I was under the influence of fig. 174. The type locality of *Alsophis nicholsi* is Buck or Capella Islands just off St. Thomas. The specimens I took on Water Island approached *nicholsi* in general appearance.

The type of *Alsophys nicholsi* is MZUM No. 80648; paratypes 80640, 80641 and MCZ No. 46503. This form should henceforth be called *Alsophys nicholsi nicholsi* Grant. The type locality and range is Buck or Capella Islands[1] off St. Thomas, Virgin Islands.

The population occurring on St. Thomas and the Virgin Islands, excepting Anegada and St. Croix and the islands and islets east of Puerto Rico, excepting Vieques which is said to have had *portoricensis*, should henceforth be known as: *Alsophis nicholsi richardi* new subspecies.

Alsophis nicholsi richardi[2], new subspecies

Type:—USNM 66522; E. Sebastian collector; St. Thomas, V.I.; 1923, male.

Paratypes:—USNM 12403A, 12403B, A. H. Riise collector; St. Thomas, V. I.

Diagnosis:—A 19 scale row *Alsophis* bearing a broken row of particolored

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[1] There are several islets called "Buck Island" among the Virgin Islands. Buck, meaning goat, is probably an influence left by the Dutch inhabitants of the Islands. It was customary to release goats on islets and capture or shoot the increase for food. Off St. Thomas lie two tiny islets nestling together like two commas with their tails separated by only about 20 feet of shallow water. Passing by these islands one would ordinarily think of them as a single island. On the charts these specks are named Capella because they are twin islets—Capella being the name of a twin star in the heavens. The fact that "Capella" means small or young goat is purely coincidental to the local name of Buck. Therefore the proper names of these rocks are Buck Island or Capella Islands.

[2] Named in honor of the original collector. It is regrettable that the two genitives should occur in the name, but I prefer to honor Richard rather than to adhere to euphony.

scales on the 5th row from the neck to a varying distance along the body; usually particolored scales above fifth row particularly at nape, occasionally extending some distance posteriorly on the eighth row. Closest to *A. anegadae*, which is a smaller, pale form with scale pores reduced in numbers and having frequently a preponderance of single pores and dark flecks on scales above the fifth row; differs from *A. nicholsi nicholsi*, which has no lateral pattern or a greatly reduced one, and from *A. portoricensis*, which bears a reticulated pattern.

SUMMARY

Brongersma studied three cotypes of *Psammophis antillensis* Schlegel, which represented three species. From these he desired to select a lectotype in order to restrict *Asaophis antillensis* (Schlegel) to a single species.

Acting on his right to select any of the three, he selected a Guadeloupe specimen to be the lectotype of *Alsophis antillensis* (Schlegel).

His action left the Virgin Islands area population, which had long been known as *A. antillensis*, without a name, unless as Schmidt claimed, *A. anegadae* Barbour and the Virgin Islands area population were identical. If this were so, the entire population would become *A. anegadae*.

The identity of the Anegada and Virgin Islands area populations has not been proven and there is good reason to believe them distinct.

Alsophis nicholsi Grant, with Buck or Capella Islands as the type locality becomes *Alsophis nicholsi nicholsi* Grant, with intergrades on Water Island.

The population on the remaining Virgin Islands, excepting Anegada and St. Croix, and on the islands and islets east of Puerto Rico, excepting Vieques, becomes *Alsophis nicholsi richardi* Grant.

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FERTILIZER REQUIREMENTS OF COFFEE GROWN ON CATALINA CLAY IN PUERTO RICO

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INTRODUCTION

Coffee is the second most important crop in Puerto Rico. About twenty per cent of the population obtain their livelihood from it. Yet, the coffee production per unit of area is very low: less than two hundred pounds of marketable coffee per acre.

Among the more obvious reasons for the low production per acre of Puerto Rican coffee, one should note that the local variety grown of *Coffea arabica* has a relatively low yield, although its berries produce a quality liquor of excellent aroma and taste, greatly preferred in the European and Cuban market. Added to the loss of this specialized market is the heavy damage inflicted by recurrent hurricanes, which has prevented the proper renovation of the coffee and shade trees and the adoption of somewhat more costly but more effective methods of cultivation. The main portion of the coffee area is on soils of the Catalina, Alonso, Los Guineos and Cialitos series, which are acid and quite low in their base exchange capacity. Because of lack of adequate fertilizer experiments on these soils, coffee growers do not know the most effective and desirable fertilizer applications to obtain maximum yields of this crop.

In his bulletin on fertilizers for coffee, McClelland (2) presented the results obtained in a series of fertilizer studies with coffee carried out at the Federal Agricultural Experiment Station at Mayaguez and elsewhere on the Island. In the summary, he made the following statements:

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1. "The production over an 8-year period showed that potash was effective in increasing yield, and that this was true particularly where nitrogen was used in addition to potash." (2, p. 32.)
2. "Growth and yield failed to show that the addition of phosphoric acid was of benefit." (2, p. 32.)
3. "Until further evidence is obtained on this point, it is believed that a fertilizer for coffee should run proportionally high in potash, such, for example, as one obtained by mixing ammonium sulphate and potassium sulphate in equal parts by weight and containing approximately 10 per cent nitrogen and 24 per cent potash." (2, p. 33.)

In order to obtain more information relative to the fertilizer requirements for coffee in Puerto Rico, a fertilizer test was started by Mr. Vicente Medina on the farm of Mr. Juan Esteva, at Lares, on January 12, 1932. This experiment was carried out as originally outlined until the seventh crop was harvested in 1939, when, as a result of a study of the yield data of the first six crops, it was decided to alter the procedure in use up to that time in the check plots. Thus, the plots that had received fertilizer applications for the first seven crops, were treated similarly for the eighth and ninth crops, the last ones of this experiment; but for these last two crops, fertilizer applications were given to some of the check plots which had received no fertilizer applications for the first seven crops.

As a result of the study of the first six crops of this experiment, another fertilizer test with coffee was started at Mayaguez. Only three crops were harvested in this experiment due to the sale of the farm on which the experiment was established, and that the new owner needed the land for other purposes.

A description of the treatments tested in these experiments and the results obtained follows in detail.

EXPERIMENT ON "CATALINA CLAY" AT LARES

Table 1 presents the treatments tested and the mean yields obtained in the plots that received the same fertilizer applications for the nine crops of the experiment established at Lares, on "Catalina Clay." In this experiment, the plots consisted of ten trees each, planted at a distance of eight feet between adjacent trees. Each plot was, therefore, 640 square feet in area, or approximately $\frac{1}{68}$ of an acre. The plots were arranged in a randomized block layout with ten replications. Five check plots, receiving no fertilizer applications, were included in each block of plots of the experiment. In table 1, however, the yields of those check plots that continued as such until the end of the test are the only ones presented.

In 1939, as has already been mentioned in the *Introduction*, a statistical study of the results obtained in the first six crops of this experiment was

made. As a result of that study, the following conclusions (1) were derived:

"The fact that no difference between the mean yields of treatments 'D', 'I' and 'J' exceeds the critical value for significance at the five per cent point, shows that potash applied in excess of 15 units did not increase significantly the yields, when the crop received in addition only five units

TABLE 1

Mean yields in hundredweights market coffee per acre of the fertilizer test performed at Lares for the nine crop cycle: 1933–1941

	TREATMENTS			REPLICATIONS										
Letter	NH₃	P₂O₅	K₂O	1	2	3	4	5	6	7	8	9	10	Total
	units	*units*	*units*											
A	5	5	15	2.47	4.23	3.60	3.68	3.51	4.73	4.62	3.00	5.89	3.91	39.64
B	10	5	15	3.56	3.61	3.22	3.61	4.03	5.66	4.60	5.55	5.47	3.76	43.07
C	15	5	15	4.03	5.09	3.25	4.75	5.18	4.75	4.75	6.47	6.13	4.05	48.45
D	5	0	15	2.89	4.84	3.27	2.92	4.53	3.89	3.56	3.56	3.30	3.42	36.18
E	5	10	15	3.14	4.02	2.84	3.78	3.38	4.07	6.62	6.00	2.76	4.43	41.04
F	5	15	15	3.16	3.51	3.65	3.75	5.35	5.65	5.36	4.67	4.82	5.06	44.98
G	5	5	20	4.03	2.97	4.10	2.70	4.54	4.57	3.57	5.19	4.32	4.00	39.99
H	5	5	25	4.89	2.92	3.05	3.55	5.19	3.44	3.91	4.89	4.89	4.73	41.46
I	5	0	20	3.80	3.74	3.28	3.65	5.60	4.72	2.82	5.18	4.10	4.07	40.96
J	5	0	25	4.65	2.59	2.76	2.81	5.48	3.80	4.68	3.12	4.19	5.47	39.55
K	0	0	0	3.41	2.54	2.42	2.38	3.80	2.90	3.31	4.22	2.23	3.17	30.38
Total............				40.03	40.06	35.44	37.58	50.59	48.18	47.80	51.85	48.10	46.07	445.70

Mean

(8) 3.964		(7) 3.999
(3) 4.307		(4) 4.146
(1) 4.845		(6) 4.096
(10) 3.618		(9) 3.955
(5) 4.104		(11) 3.038
(12) 4.498		
		44.570

Notes: 1 unit = 7.5 pounds of substance per acre. Values in italics are estimated.

of nitrogen. A similar conclusion is derived on comparing the mean yields of treatments 'A', 'G' and 'H', when the crop received five units each of nitrogen and phosphoric acid. Therefore, on this basis, it can be concluded that applications of potash at rates higher than the minimum 15 units used in this experiment were not effective in increasing the yields over the yield produced by the minimum 15 units of potash used.

"Neither five nor 10 units of phosphoric acid were sufficient to increase significantly the yields, when the crop received in addition five units of nitrogen and 15 units of potash. This conclusion is based on a comparison of the mean yields of treatments 'D', 'A', and 'E'. The application of 15 units of phosphoric acid in treatment 'F', however, produced a significant increase in yield when compared with the no-phosphoric acid application of treatment 'D'.

"Neither 10 nor 15 units of nitrogen produced any significant increases in yield over that produced by five units of nitrogen, when the crop received in addition five units of phosphoric acid and 15 units of potash. This is deduced from a comparison of the mean yields of treatment 'A', 'B', and 'C'.

"The tendency of the yields, however, is to increase with increasing amounts of both nitrogen and phosphoric acid. This is in full accord with the idea that the yield of a crop depends on the concentration of nutrients in the soil, and that the more nutrients, the higher the yield up to an optimum point. A small application of some nutrient may not prove its effectiveness in increasing the yield due to the heterogeneity of the soil, while a larger amount of the same nutrient may prove effective in so doing. This behaviour cannot be interpreted as indicating that the small application has no effect while the large application has a real effect. On the contrary, both applications are effective, only that the small one is not sufficiently effective to influence the yield statistically under the conditions in which the experiment is performed. This has happened in this case with the applications of phosphoric acid where the application of both five and 10 units did not produce a statistical increase in yield, while the 15-unit application did produce it.

"The applications of nitrogen were, according to the statistical analysis, not significant, but when used in conjunction with the application of five units of phosphoric acid—which had not demonstrated any significant effect by themselves—produced significant increases in yield.

"The applications of potash, beyond the minimum 15 units used, however, have produced no significant increases. This is not to be interpreted in the sense that potash is not necessary for high coffee yields, but in the sense that the nutrient requirements of the crop had been fulfilled with an amount of potash which was no larger, and may have been smaller, than the soil content plus the 15 units applied as minimum.

"If when the project is closed, the results are the same as those obtained to date, the recommendation would have to be in favor of the use of a fertilizer analysis containing the maximum amounts of nitrogen and phosphoric acid and the minimum amount of potash used in this test, that is, an application of about 112.5 pounds per acre each of ammonia, phosphoric

acid and potash. At that time there would be no evidence on which to recommend the use of a smaller amount of potash, since this range of potash application has not been investigated.''

It must be pointed out that the use of the small amounts of phosphoric acid and the large amounts of potash tested in this experiment were due to the results obtained by McClelland (2) mentioned above. The results at the date of this study indicated, however, trends altogether different from those obtained by McClelland (2), and, accordingly, from the results expected at the time that the experiment was started.

The statistical analysis of the yield data obtained in the whole nine-crop cycle appears in table 2, and the results of the evaluation of the statistical significance of the yield differences that may be attributed to differences in the rates of application of the fertilizer substances are presented

TABLE 2

Analysis of the total sum of squared deviations of the data of table 1

SOURCE OF THE DEVIATIONS	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE ESTIMATE	F
Blocks..............................	9	27.3257		
Treatments..........................	10	21.4278	2.1428	3.30**
Error...............................	87	56.5363	0.6498	
Total.............................	106	105.2898		

There are highly significant differences between the treatment means.
Values to be exceeded for significance between two 10-plot means:
  At the 5% point.......... 0.717 hundredweights market coffee per acre
  At the 1% point.......... 0.950 hundredweights market coffee per acre

in table 3. This table indicates that the conclusions to be derived from the results of the whole nine-crop cycle are about the same as were derived from the interpretation of the results of the first six crops of the experiment. The effect of the application of the 15 units of the nitrogen has now proved to be significant: a conclusion that was suggested but not verified by the previous study.

The lack of response to applications of potash in excess of the minimum application of 15 units, or 112.5 pounds of potash per acre, suggested the possibility of maintaining the crop yields with smaller applications of potash. To test this possibility, forty of the fifty plots which had received no fertilizer applications for the first six crops were selected for the determination of the effects on the crop yields of applications of potash below the minimum used up to that time. The treatments used in these

plots, and the results obtained in the two crops in which said treatments were tried, are presented in table 4.

Table 5 shows the results of the statistical analysis of the yield data of table 4. In table 5 it may be seen that the differences between the mean yields of the different treatments are not significant.

It should be noted that this lack of response to the potash applications was observed in plots which had received no potash applications for the previous seven crops. The conclusion that the potash applications did not increase the crop yields at the experimental site, under the conditions

TABLE 3

Significance of differences between the mean yields obtained with different amounts of application of each fertilizer substance

FERTILIZER SUBSTANCE	TREATMENT COMPARISON	DIFFERENCE BETWEEN MEAN YIELDS	REMARK AS TO SIGNIFICANCE OF DIFFERENCE
Nitrogen	B–A	0.343	Not significant
	C–B	0.538	" "
	C–A	0.881	Significant at 5% point
Phosphoric acid	A–D	0.346	Not significant
	E–A	0.140	" "
	F–E	0.394	" "
	E–D	0.486	" "
	F–A	0.534	" "
	F–D	0.880	Significant at 5% point
Potash	I–D	0.478	Not significant
	J–I	−0.141	" "
	J–D	0.337	" "
Potash	G–A	0.035	Not significant
	H–G	0.147	" "
	H–A	0.182	" "

and for the duration of the experiment, appears to be warranted by the above results. This conclusion is also in contrast with what was to be expected from McClelland's results (2).

EXPERIMENT WITH "CATALINA CLAY" AT MAYAGUEZ

The experiment at Lares started with 16-year-old trees. On that account, data on the fertilizer requirements of young coffee trees were still lacking. To obtain desired information, the other experiment mentioned in the *Introduction* was started at Mayaguez.

The latter experiment was established on a private farm at Km. 6.1 of

road No. 13 from Mayaguez to Las Marías, with six-year-old trees of the Puerto Rican variety of *Coffee arabica*. The soil of the experimental field was also a "Catalina Clay" and the trees were planted, as in the former experiment, at a distance of eight feet between adjacent trees. "Guaba" *Inga Inga* (L) Britton, and "guama" *Inga laurina* (SW) Wild, were used to provide the shade. The plots of this experiment consisted of 16 trees each, so that each plot was 1024 square feet, or approximately $1/42.54$ of an acre, in area. Each treatment was replicated seven times. The

TABLE 4

Mean yields in hundredweights market coffee per acre of the fertilizer test performed at Lares for the two crop cycle: 1940–41

TREATMENTS				REPLICATIONS										
Letter	NH₃	P₂O₅	K₂O	1	2	3	4	5	6	7	8	9	10	Total
	units	units	units											
L	15	15	0	3.43	3.06	1.69	2.87	3.30	3.32	3.65	4.27	6.10	3.73	35.42
M	15	15	5	3.06	3.57	3.19	2.95	4.72	3.37	5.00	3.91	4.50	4.22	38.49
N	15	15	10	2.54	3.56	3.32	3.39	5.65	3.89	3.38	4.55	8.00	4.13	42.41
O	15	15	15	3.92	3.16	2.91	3.25	3.68	3.17	4.72	3.30	3.08	4.84	36.03

Note: 1 unit = 7.5 pounds of substance per acre.

TABLE 5

Analysis of the total sum of squared deviations of the data of table 4

SOURCE OF THE DEVIATIONS	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE ESTIMATE	F
Blocks	9	21.8684		
Treatments	3	3.0195	1.0065	1.32
Error	27	20.5625	0.7616	
Total	39	45.4504		

The differences between the treatment means are not significant.

eleven treatments tested and the results obtained in three crops harvested in this experiment are presented in table 6. The fertilizers were applied in a narrow band six inches deep, just beneath the drip of the trees and around them. In cases where the land was too steep; the band was made in a half-moon shape on the upper side of each tree. Only one fertilizer application was made for each crop, during January, after the yearly harvest.

Table 7 shows the result of the statistical analysis of the yield data of

table 6. It shows that there were significant differences between the mean yields of the treatments.

Table 8 presents the results obtained in the evaluation of the statistical significance of the yield differences that may be attributed to differences in the rates of application of the different fertilizer substances. In it, one

TABLE 6

Mean yields in hundredweights of market coffee per acre of the fertilizer test performed at Mayagüez for the three crop cycle: 1942–44

TREATMENTS				REPLICATIONS								MEAN
Letter	NH₃	P₂O₅	K₂O	1	2	3	4	5	6	7	Total	
	pounds per acre											
A	100	0	0	2.38	2.42	2.67	1.59	1.41	1.03	1.39	12.89	1.841
B	100	100	0	1.75	2.20	3.42	1.91	1.69	2.97	2.92	16.86	2.409
C	100	0	100	2.49	2.98	1.72	0.61	1.88	2.15	2.79	14.62	2.089
D	0	100	100	3.22	2.65	3.59	2.82	1.68	1.56	1.23	16.75	2.393
E	0	0	100	3.09	1.96	1.34	2.56	1.17	2.60	1.98	14.70	2.100
F	0	100	0	5.13	1.79	2.36	2.50	1.65	2.40	2.55	18.38	2.626
G	100	100	100	2.57	2.90	3.11	2.47	1.08	2.49	1.94	16.56	2.366
H	200	100	100	2.57	2.39	2.98	1.72	0.95	1.89	2.84	15.34	2.191
I	100	200	100	5.78	2.52	3.22	3.07	2.38	4.96	1.31	23.24	3.320
J	100	100	200	2.98	3.64	1.09	3.15	2.32	4.46	3.11	20.75	2.964
W	0	0	0	1.71	2.88	1.89	0.47	1.08	1.72	1.42	11.17	1.596

TABLE 7

Analysis of the total sum of squared deviations of the data of table 6

SOURCE OF THE DEVIATIONS	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE ESTIMATE	F
Treatments..........................	10	16.6823	1.6682	1.99*
Error...............................	66	55.1900	0.8362	
Total..............................	76	71.8723		

There are significant differences between the treatment means.

Values to be exceeded for significance between two 7-plot totals:

    At the 5% point.......... 0.976 hundredweights market coffee per acre

    At the 1% point.......... 1.297 hundredweights market coffee per acre

may see that the only fertilizer substance that has affected the yields in a significant way has been phosphoric acid. This caused significant increases when applied at the rate of 100 pounds P_2O_5 per acre, in the absence of applications of nitrogen and potash, and also when applied at the rate of 200 pounds P_2O_5 per acre, in the presence of applications of 100 pounds

each per acre NH_3 and K_2O. In all the other cases, increases in the amounts of phosphoric acid applied were associated with increases in the crop yields, though in only one of the other four cases did this increase approach significance. In none of the cases were the yields affected significantly by increases in the amounts of nitrogen and potash applied. For increases in crop yield in this field during the period covered by this test, therefore, the phosphoric acid applications proved to be necessary,

TABLE 8

Significance of differences between the total yields obtained with different amounts of application of each fertilizer substance

FERTILIZER SUBSTANCE	TREATMENT COMPARISON	DIFFERENCE BETWEEN YIELDS	REMARK AS TO SIGNIFICANCE OF DIFFERENCE
Nitrogen	A–W	0.245	Not significant
	B–F	−0.217	" "
	C–E	−0.011	" "
	G–D	−0.027	" "
	H–G	−0.175	" "
	H–D	−0.202	" "
Phosphoric acid	F–W	1.030	Significant at 5% point
	B–A	0.568	Not significant
	D–E	0.293	" "
	G–C	0.277	" "
	I–G	0.954	" "
	I–C	1.231	Significant at 5% point
Potash	E–W	0.504	Not significant
	C–A	0.248	" "
	D–F	0.233	" "
	G–B	−0.043	" "
	J–G	0.598	" "
	J–B	0.555	" "

whereas the nitrogen and potash did not exert significant effects on the crop yields.

CONCLUSIONS

Two fertilizer experiments at Lares and Mayaguez were conducted on "Catalina Clay" with the Puerto Rican variety of *Coffee arabica*.

The experiment at Lares indicated that, for maximum coffee yields, nitrogen and phosphoric acid applications were required. The experiment at Mayaguez indicated that, for maximum coffee yields, phosphoric acid applications were necessary. The experiment at Mayaguez lasted

for only three crops, however, and it should be pointed out that though the beneficial effect of the nitrogen applications on the crop yields at Lares were not statistically significant with the results of the first six crops, they were with the results of the whole nine-crop cycle.   Had the experiment at Mayaguez lasted long enough, the nitrogen applications might have proved to be essential for maximum crop yields.   It should be remarked that the shade trees are leguminous and, therefore, they may contribute to supply at least a portion of the crop's nitrogen requirements.

Since, however, these experiments represent but two localities, further work should be done to determine the fertilizer requirements in other sections, at other altitudes, and in some of the other important soil types as regards coffee production.

SUMMARY

The results obtained in two coffee fertilizer tests performed with the Puerto Rican variety of *Coffea arabica* on "Catalina Clay" are presented, statistically analyzed, and discussed.

Nitrogen and phosphoric acid applications seem to be of greater importance as regards market-coffee production of the above variety in the soil type used, than are the applications of potash, which had no significant effects on the yields.

These results are in sharp contrast with the results obtained by McClelland, who found potash applications to be essential and phosphoric acid applications to be not essential for maximum coffee production in Puerto Rico.   It should be noted that McClelland's experiments were carried out on other soil types, which were probably not in condition to provide the coffee trees with their potash requirements.

RESUMEN

Los resultados obtenidos en dos experimentos de abono realizados con la variedad Puerto Rico de *Coffea arabica* en el suelo Catalina arcilloso han sido presentados, analizados estadísticamente y discutidos.

Las aplicaciones de nitrógeno y ácido fosfórico parecen ser más importantes en cuanto se refiere a la producción de café comercial de la variedad Puerto Rico en el suelo Catalina arcilloso que las aplicaciones de potasa, las cuales no demostraron tener efectos significativos en dichos rendimientos.

Estos resultados difieren radicalmente de los resultados obtenidos por McClelland, quien encontró que las aplicaciones de potasa eran esenciales, y que las de ácido fosfórico no eran necesarias para la producción máxima de café en Puerto Rico.   Debe llamarse la atención al hecho de que McClelland realizó sus experimentos en otros suelos, los cuales probablemente no

se hallaban en condiciones de proveer al cafeto la potasa requerida por el mismo.

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TRACING THE MINERAL FROM THE SOIL TO THE PLANT TO THE ANIMAL BLOOD

PART I. EFFECT OF LIME ON THE MINERAL COMPOSITION OF THE SOIL, OF THE GRASS, AND ON THE CROP YIELD

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The land in pasture, fallow, and idle in Puerto Rico, is estimated by the 1940 Census of Agriculture to be around 776,103 "cuerdas," of which the amount of acid land dispersed in the humid area may be estimated to be about 69.3 per cent or 537,696 "cuerdas." (1 cuerda = 0.971 acre)

How the application of lime to these soils would influence the mineral composition of the soil and of the grass is not known. According to Beeson (1), fundamental studies are lacking of what changes take place in the soil when the fertilizers are supplied, and on what effect these changes will have on the plant.

This paper reports the effect that a calcium application to an acid soil has on the composition of calcium, phosphorus, magnesium, manganese, and iron of the soil, of the grass grown in this soil, and on the yield of this crop.

EXPERIMENTAL WORK

Eighteen plots, each with an area of four-tenths of an acre, were selected in a field of "Fajardo clay" at the Experiment Station Farm at Río Piedras. "Fajardo clay" is an acid red soil of the humid region, derived from old, high alluvial material and from outwash fans of adjacent shale hills. The relief is level or gently sloping.

Limestone was added on June 25, 1943 to half of the randomized plots at the rate found by the lime-requirement test reported by Riera (4). The amount of limestone applied varied from 8 to 10 tons per acre. The field was planted in the middle of July 1943 with a mixture of Para grass *Panicum purpurascens*, and Carib grass *Eriochloa polystachya*, the former known as "Malojillo" and the latter as "Malojilla". Para and Carib grasses comprise the most valuable pasture and soilage grasses in the lowlands of the northeastern part of Puerto Rico.

Five consecutive crops were harvested on the dates reported in table 1. These dates varied for each crop because the grasses were cut daily, in strips, to supply to the stable herd. The grass from each strip was weighed in the field. The third and fifth crops were fertilized with ammonium sulphate at the rate of 500 pounds per acre. From January 29 to September 15, 1945, grass from the third to fifth crops inclusive, was supplied

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daily to fifteen female goats used in a supplementary experiment to find the effect of the chemical composition of this grass on their health.

METHODS OF ANALYSES

SOILS

Three composite samples of the soil were taken from each plot; the first in June 1943 previous to the lime application; the second and the third, in September 1944 and May 1945, fifteen and twenty-three months, respectively, after the lime application. Each soil sample was analyzed for pH and for exchangeable calcium, magnesium, manganese, and for available iron and phosphorus.

Exchangeable Calcium, Magnesium, Manganese. Exchangeable calcium, magnesium, and manganese, were run by Peech's (3) method as follows: Weigh 10 grams of air-dried soil and leach into a 400 ml. beaker with about 225 ml. of normal neutral ammonium acetate solution. Dry leachate carefully in a hot plate and destroy organic matter and ammonium salts, adding 5 ml. of fuming nitric acid and 1 ml. of concentrated sulphuric acid and warming until the reaction has subsided and the brown fumes are no longer given off. Cool and rinse. Evaporate to dryness at low heat and continue heating for about 10 minutes to dehydrate the salts. Place the beaker in an electric muffle at 150°–200°C. and heat to 380°C. and hold at this temperature for 10–15 minutes. Treat residue with 3 ml. of 1:1 hydrochloric acid to dissolve the oxides of manganese and iron. Evaporate to dryness on steam bath and continue heating for fifteen minutes to dehydrate silica. Dissolve the salt residue with 10 ml. of 0.1 normal nitric acid. The solution should be colorless and clear, except for a trace of silica, which is either allowed to settle out in the beaker or centrifuge if necessary in a 15 ml. centrifuge tube. The solution from the beaker is decanted into a 15 ml. test-tube. This is solution A.

Transfer 2 ml. aliquot of solution A, equivalent to 2 grams of soil, to a 15 ml. centrifuge tube for the determination of calcium and magnesium. Add 0.2 ml. of ferric chloride solution (1 ml. = 1 milligram Fe), 3 ml. distilled water, and 2 ml. of 10 per cent sodium acetate solution. Mix and add 1 ml. of 0.1 normal sodium hydroxide, and mix again. Place the centrifuge tube in a water bath kept at 95°C. Add 1 ml. of a saturated solution of bromine, and maintain water bath temperature for at least one hour to flocculate the manganese dioxide, and to expel the excess of bromine. Add 2 ml. of 25 per cent ammonium chloride solution and digest for about 15 minutes. Add a drop of methyl red; and if the color of the indicator persists, indicating complete expulsion of bromine, remove the

tube from the water bath, cool, add 0.6 normal ammonium hydroxide from a burette until the color of the solution changes from a slightly red to a deep yellow; add 2 drops in excess. In general, it usually requires 0.5 ml. of 0.6 normal ammonium hydroxide. Make up to a volume of 13 ml. with water and add 5 drops of water in excess to allow for evaporation. Mix with a stirring rod and digest in water bath at 80°C. for 5 minutes to flocculate the precipitate. Centrifuge *while hot*, for 10 minutes. Designate as solution B.

Calcium. Pipette 10 ml. of solution B. equivalent to 1.5385 grams of soil, without disturbing the manganese-iron-aluminum precipitate, into a 15 ml. centrifuge tube. This is done best by holding the tube in front of a mirror. Add 0.5 ml. of 0.5 normal hydrochloric acid and 0.9 ml. of water and place in a water bath at 70°C. Mix by spinning the tube, add 2 ml. of 3 per cent ammonium oxalate. Mix thoroughly again and digest for 30 minutes at 70°C. Remove the tube from the bath and let stand for 30 minutes. The volume of the solution at this point is 13.4 ml. The excess of 0.4 ml. evaporates and the final volume of the solution is 13 ml. Decant the clear supernatant liquid into a dry test tube and keep the test-tube inverted at an angle of 45 degrees for a few minutes. Save the liquid for the magnesium determination, (Solution C). The precipitate of calcium oxalate remains in the test-tube. Add to the precipitate, 5 ml. of 2 normal ammonium hydroxide solutions saturated with calcium oxalate, break up the precipitate with a stirring rod, wash the rod, and centrifuge for 15 minutes at 1700 r.p.m. Decant the solution, drain the tube, and discard the clear liquid. Wash again, and centrifuge, if necessary. Dissolve the precipitate with 5 ml. of ten per cent sulfuric acid solution. Heat to 70°C. in a water bath and titrate with a standard 0.025 normal potassium permanganate solution.

The amount of calcium in soil is calculated as follows:

$$\text{p.p.m. Ca in soil} = (\text{ml. KMnO}_4 \times 0.025 \times 0.02004 \times 1{,}000{,}000) \div 1.5385$$
$$= 326 \times \text{ml. KMnO}_4$$

Magnesium. Take 10 ml. of solution C, equivalent to 1.1835 grams of soil, into a 15 ml. centrifuge tube. Place the tube in a bath at 70°C., add 0.8 ml. of 2 per cent alcoholic solution of 8-hydroxyquinoline, mix immediately by stirring, and then add 0.4 ml. of concentrated ammonium hydroxide from a buret. Stir vigorously for 1 minute, or longer if the amount of magnesium is extremely small, until full turbidity develops. Wash the stirring rod with a few drops of water and replace the centrifuge tube in a water bath at 70°C. for 10 minutes to flocculate the precipitate. If a number of magnesium determinations are to be carried out simultaneously,

set the centrifuge tubes aside after precipitation, until the magnesium in the last tube has been precipitated, then replace the tubes in a bath at 70°C. for 10 minutes. After digestion for 10 minutes, cool by immersing the centrifuge tubes in a bath at about 25°C., and allow to stand for 45 minutes to assure complete precipitation of magnesium; then add 0.5 ml. of 95 per cent ethyl alcohol slowly down the sides of the centrifuge tube, rotating the tube at the same time in order to wash down the precipitate and to form a layer of alcohol on the surface of the solution. Centrifuge for 15 minutes at 1700 r.p.m. and by using gentle suction draw off 2 to 3 ml. of the clear liquid to remove the layer of alcohol. Decant carefully and discard the solution; wipe the mouth of the tube with filter paper, add 5 ml. of ammoniacal ammonium acetate (8 ml. concentrated ammonium hydroxide in 300 ml. of 0.7 normal ammonium acetate), wash solution down the sides of the tube, break up the precipitate with a stirring rod, and wash the rod into the tube; add 0.5 ml. of alcohol down the sides of the tube to prevent creeping of the precipitate, and centrifuge for 15 minutes at 1700 r.p.m. Draw off the layer of alcohol, decant, and repeat the washing once more as directed above. Dissolve the precipitate in 4 ml. of 0.5 N hydrochloric acid, dilute to 13 ml. with water, stopper, and mix. Transfer a 1 ml. aliquot, equivalent to 0.0910 gram of soil to a 50-ml. volumetric flask, and add about 35 ml. of water, 5 ml. of 20 per cent sodium carbonate, and 3 ml. of phenol reagent, mixing the contents after each addition. Place the flask in boiling water for 1 minute, remove from the bath, and cool after 15 minutes. Make to volume, mix, and read in the spectrophotometer. The phenol reagent was prepared as follows: To 750 ml. of water in a 2-liter flask add 100 grams of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$), 20 grams of phosphomolybdic acid (20 $MoO_3 \cdot 2H_3PO_4 \cdot 48H_2O$), and 50 ml. of 85 per cent phosphoric acid. Boil for 2 hours, cool, and dilute to 1 liter with distilled water.

The transmittance-concentration curve (figure 1) for magnesium was developed as follows: Dissolve 0.15 gram of magnesium sulphate ($MgSO_4 \cdot 7H_2O$) in 100 ml. of 10 per cent ammonium chloride solution, heat to 60–70°C., add 10 ml. of the 8-hydroxyquinoline reagent, and make the solution alkaline with 4 ml. of concentrated ammonium hydroxide. Digest for 10 minutes, collect the precipitate on a fritted glass crucible, wash with hot dilute ammonium hydroxide, and dry at 140°C. Dissolve 0.0643 gram of the dried precipitate in 20 ml. of 0.5 normal hydrochloric acid and dilute to 500 ml. One milliliter contains 0.01 milligram of magnesium. Take 50 ml. of this standard solution and dilute to 100 ml. One milliliter of this second standard contains 0.005 milligrams of magnesium. The following transmittances were obtained, in a Coleman spectropho-
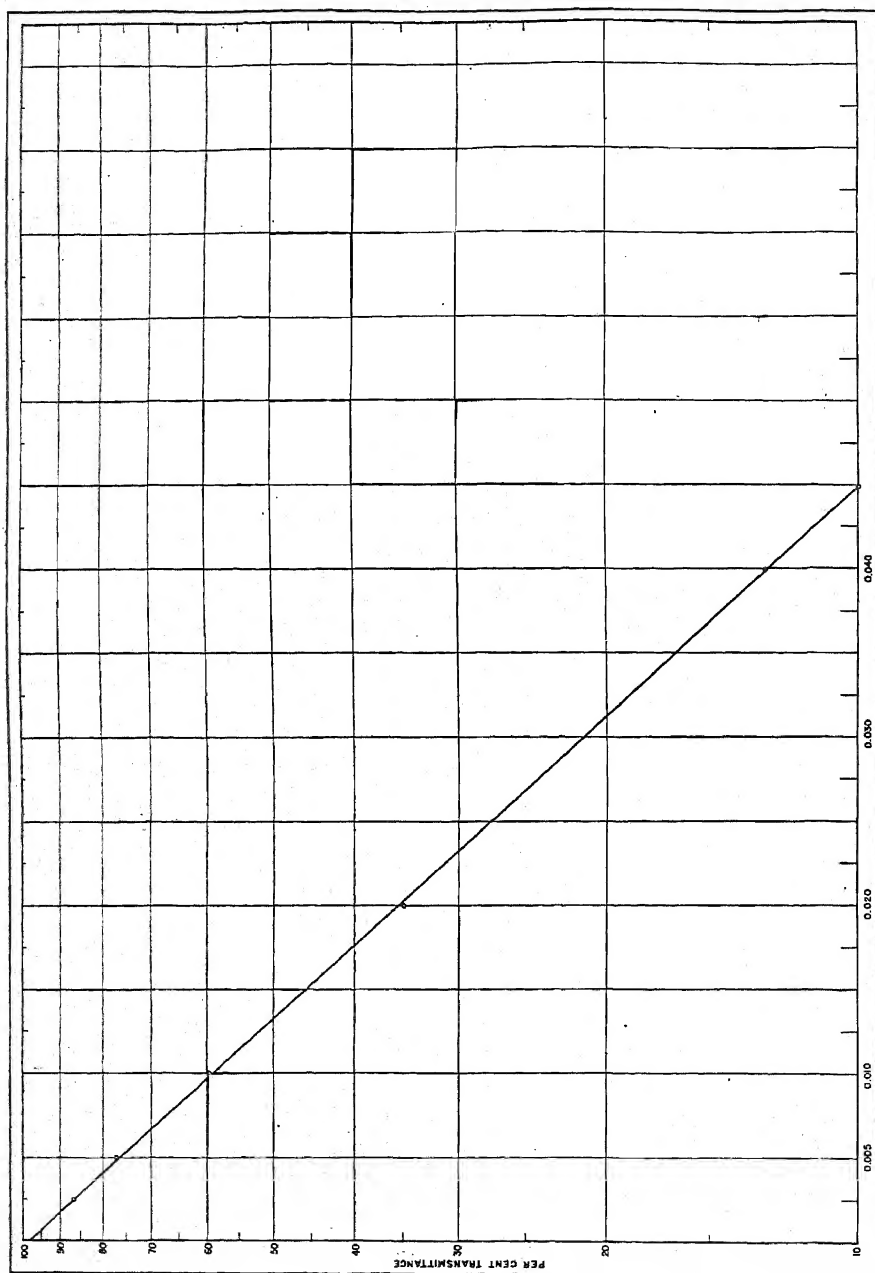
FIG. 1   Available magnesium in Soils and total magnesium in plants.   Abscissa represents milligrams of magnesium as Mg.

tometer, model 11, using a PC-4 filter and a wave length of 650 mμ (figure 2), and a reagent blank as reference solution:
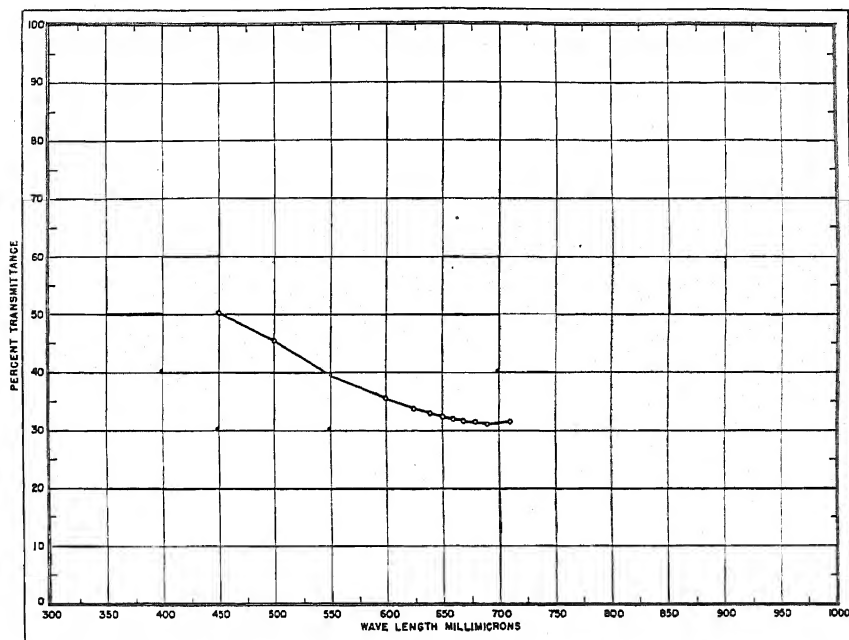
FIG. 2   Spectral-Transmittance curve for magnesium, as per method for soils and plants.

STANDARD MAGNESIUM SOLUTION		TRANSMITTANCE
ml.	mg. Mg	%
0.5	0.0025	87.0
1	0.005	77.2
2	0.010	59.8
4	0.020	35.1
8	0.040	12.9
10	0.050	8.9

The color was developed as explained in the procedure.

The amount of magnesium in soil is calculated as follows:

$$\text{p.p.m. Mg in soil} = \frac{\text{milligrams Mg in curve} \times 1{,}000{,}000}{1{,}000 \times 0.0910}$$

$$= \text{mgm. Mg} \times 10{,}989$$

Manganese.   Manganese was determined by the simplified periodate method described by Peech (3).   Transfer 2 ml. of solution, equivalent to 2 grams of soil, to a test-tube graduated at 11 ml.   Add 1 ml. of 85 per cent

phosphoric acid, dilute to 11 ml. with water, and add 0.3 ml. to allow for evaporation, and mix with a stirring rod.   Place in a water bath at 95°C., add about 50 milligrams of sodium periodate, mix thoroughly again, and leave in the bath for 1 hour to assure full color development.   Cool, make to volume if necessary; mix and read in the spectrophotometer.

The transmittance-concentration curve (figure 3) for manganese was developed as follows: To 22.8 ml. of 0.1 normal potassium permanganate solution in a 250 ml. Erlenmeyer flask, add about 50 ml. of water and a few drops of concentrated sulfuric acid.   Heat to boiling and reduce the permanganate by the addition of sodium sulfite until the solution is colorless. Boil off the excess of sulfur dioxide and dilute to one liter.   One milliliter of this solution is equivalent to 0.025 milligrams of Mn.   The following transmittances were obtained in a Coleman spectrophotometer, model 11, using a PC-4 filter and a wave length of 525 mμ (figure 4) and a reagent blank as reference solution:

STANDARD MANGANESE SOLUTION		TRANSMITTANCE
ml.	mg. Mn	%
1	0.025	79.8
2	0.050	63.6
3	0.075	51.0
5	0.125	33.5
8	0.200	19.1
10	0.250	13.9

The color was developed as explained in the procedure.

The amount of manganese in soil is calculated as follows:

$$\text{p.p.m. Mn in soil} = \frac{\text{milligrams Mn in curve} \times 1,000,000}{1,000 \times 2}$$

$$= 500 \times \text{mg. Mn in curve}$$

Available Phosphorus and Iron.   Available phosphorus and iron in the soil were extracted with Morgan's Universal extracting solution, normal sodium acetate buffered at pH 4.8 with acetic acid as follows: 12.5 grams of air-dried soil and 25 ml. of extracting solution were placed in a test-tube, 6″ long and 1″ in diameter.   The tube was stoppered and shaken horizontally for 2 minutes, in a reciprocating shaker (Amer. Instrument Co. cat. #7-155) at a speed of about 120 shaking cycles per minute.   The extract was filtered in a Whatman filter paper No. 1.

Available Phosphorus—Phosphorus was precipitated as ammonium phosphomolybdate, reduced to the blue color with aminonaphtholsulfonic acid and determined colorimetrically as per Wolf's (5) procedure as follows: Take an aliquot of 5 ml. of soil extract, equivalent to 2.5 grams of soil,
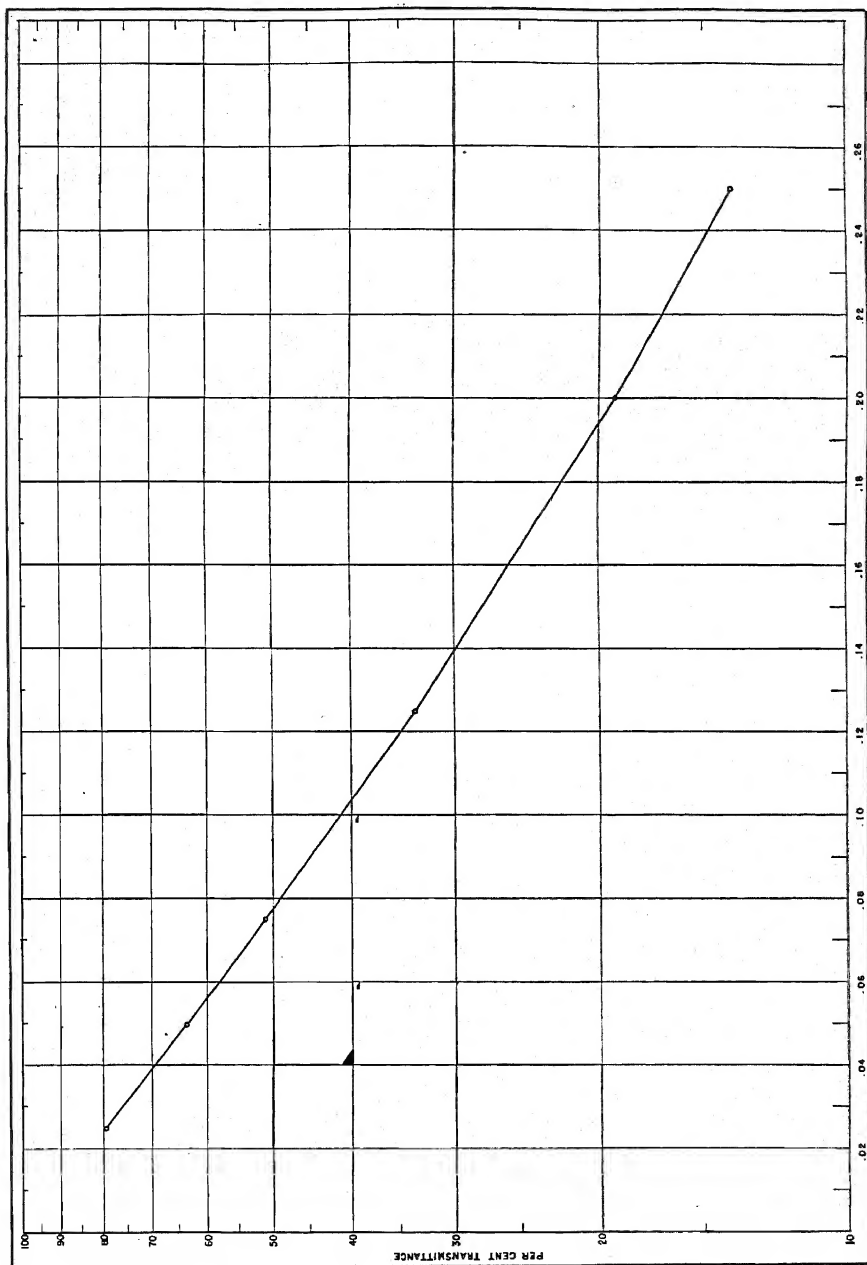
FIG. 3   Available manganese in soils and total manganese in plants.   Abscissa represents milligrams of manganese as Mn.
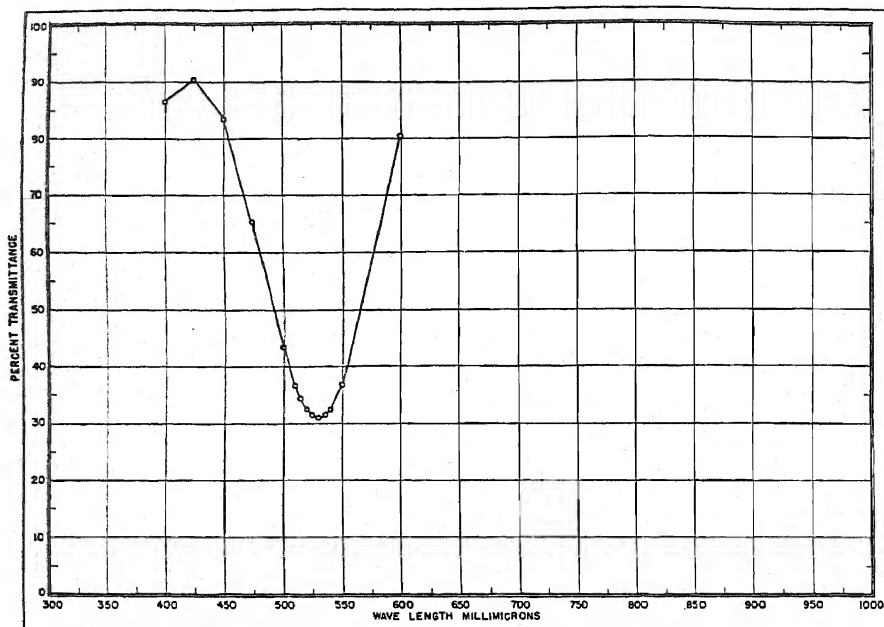
Fig. 4   Spectral-Transmittance curve for manganese as per method for soils and plant
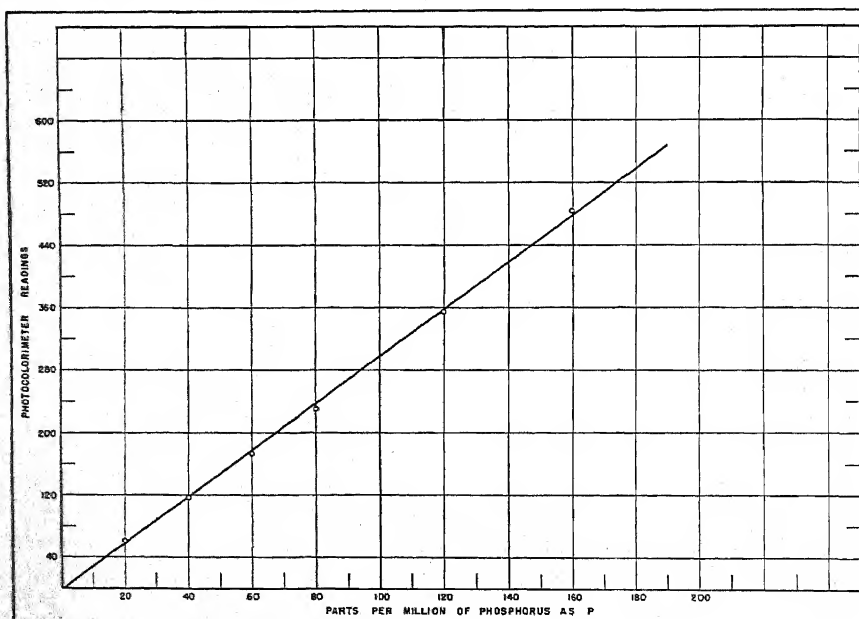
Fig. 5   Available phosphorus in soils.

dilute to 20 ml. with extracting solution, add 4 ml. of ammonium molybdate solution (2.5 per cent in 6 normal sulfuric acid, and 2 ml. of aminonaphthol-sulfonic acid solution (15 grams of anhydrous sodium bisulfite, are dissolved in 100 ml. of water, and 0.5 gram of pure, dry 1-amino-2-naphthol-4-sulfonic acid, and 1.5 grams of anhydrous sodium sulfite, are added; shake, make up to 500 ml. and store in a brown bottle).

The concentration curve for phosphorus (figure 5) was obtained in a Klett-Summerson photoelectric colorimeter No. 2141 with red filter 66 covering wave lengths from 640 to 700 mμ and instrument set at zero with reagent blank. The procedure was as follows: Weigh 0.1006 grams of sodium monobasic phosphate ($NaH_2PO_4 \cdot 2H_2O$) and dissolve in one liter of water. One milliliter of this solution is equivalent to 20 parts P per million. The following readings were obtained in the photoelectric colorimeter:

STANDARD PHOSPHORUS SOLUTION			PHOTOCOLORIMETER READING
ml.	mg. P	p.p.m. P	
1	0.02	20	60.5
2	0.04	40	115.4
3	0.06	60	174.4
4	0.08	80	229.9
6	0.12	120	353.5
8	0.16	160	483.0

The slope of this curve was found not to be constant. To check the slope, three phosphorus standards should be run with the unknown.

The amount of phosphorus in soil is calculated as follows:

$$\text{p.p.m. available P in soil} = \text{p.p.m. P in curve} \times \frac{1}{2.5}$$

$$= \text{p.p.m. P in curve} \times 0.4$$

Available Iron—An aliquot of 1 ml. of the soil extract equivalent to 0.5 gram of soil, was poured in a test-tube graduated at 10 ml. The color was developed as per method of Saywell and Cunningham, described by Parks et al (2), as follows: Add 1 ml. of 10 per cent hydroxylamine hydrochloride solution and 0.5 ml. of ortho-phenanthroline (1.5 per cent in 95 per cent ethanol), make to volume, mix and read in the photoelectric colorimeter. As the original extract was buffered to pH 4.8 there was no need of adjusting the pH with ammonium hydroxide as mentioned by Parks.

The concentration curve (figure 6) for iron was developed as follows: Weigh one-gram of c.p. iron wire in a liter volumetric flask and dissolve in about 150 ml. of 1:6 sulfuric acid; add 5 ml. of concentrated nitric acid as oxidizing agent; boil to expel SO_3 fumes, and complete volume to one liter.

One milliliter of this solution is equivalent to one milligram Fe.   Ten milliliters of this solution were diluted to one liter; one milliliter of this solution contains 0.01 milligrams Fe.   The following readings were obtained in a Klett-Summerson photoelectric colorimeter, No. 2141, with blue filter 42
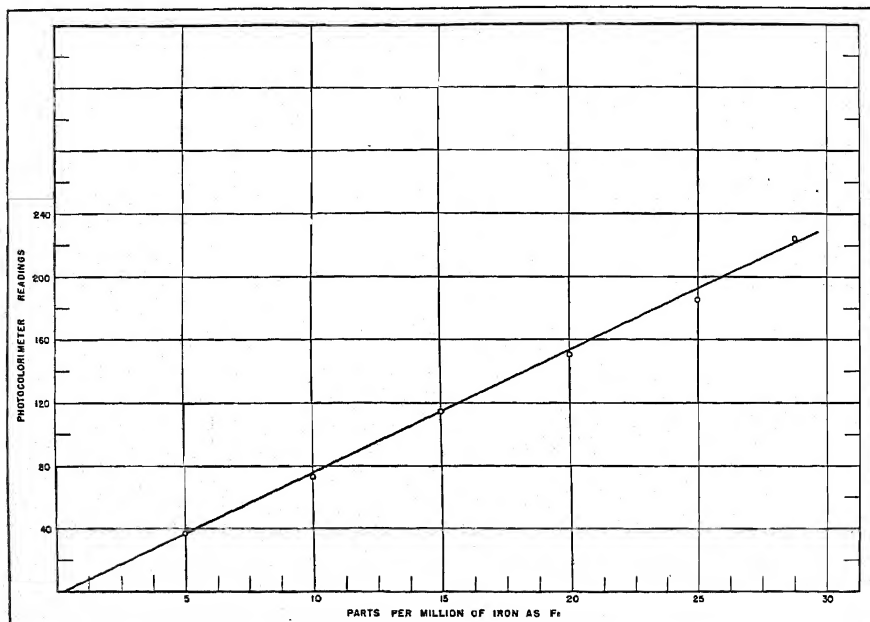
FIG. 6   Available iron in soils.

covering wave lengths from 400–465 mμ, and the colorimeter set at zero with reagent blank:

STANDARD IRON SOLUTION			PHOTOCOLORIMETER READING	SLOPE FACTOR
ml.	mg. Fe	p.p.m. Fe		
0.5	0.005	5	37	.1351
1.0	0.010	10	73	.1370
1.5	0.015	15	115	.1304
2.0	0.020	20	151	.1325
2.5	0.025	25	184	.1351
3.0	0.030	30	224	.1339

The color was developed as explained in the procedure.   The slope of the curve was found to be constant; its average value is 0.1340.

p.p.m. available Fe in soil = p.p.m. Fe in curve $\times$ 2

$$= \text{photocolorimeter reading} \times 0.1340 \times 2$$

$$= 0.268 \times \text{photocolorimeter reading}$$

GRASS

A composite sample (about ten pounds) of the standing grass was taken from each plot. The samples were dried to constant weight in a hot air oven at 150°F. Each sample was ground in a Wiley mill and sifted through a 1 mm. sieve. The ground samples were left overnight at room temperature to absorb atmospheric moisture. The dates of grass samplings are reported in table 1.

A 7.50 gram sample of dry grass was weighed in a 600 ml. pyrex beaker for each determination and the procedure of Parks et al (2), omitting the dithizone extraction, was followed as explained below.

Destruction of Organic Matter and Removal of Silica. Destroy the organic matter with nitric and perchloric acids; add first 12.5 ml. of concentrated nitric acid, place a cover glass on top of the beaker, and heat in a steam plate or hot plate at low temperature, in the hood; add again 12.5 ml. of nitric

TABLE 1

Dates of soil and grass samplings, of fertilizer application, of harvesting, and age of grass at harvest time

CROP	DATES OF SOIL SAMPLING	DATES OF GRASS SAMPLING	DATES OF AMMONIUM SULPHATE APPLICATION	HARVESTING DATES	AGE OF CROP HARVESTED	
					At start	At end
					mo.	*mo.*
First...........	6/43			1/13–27/44	5.5	6.0
Second........	9/44	8/28/44		8/28–11/14/44	7.0	9.5
Third..........		11/16/44	10/10/44	11/15/44–3/29/45	2.5	4.5
Fourth........	5/45	4/10/45		3/30–7/29/45	4.5	4.0
Fifth...........		7/16/45	4/25/45* 6/5/45*	7/30–10/25/45	4.0	3.0

* Only one application; dates refer to application for half of each plot.

acid and evaporate to near dryness. Add to residue 25 ml. of concentrated nitric acid and 25 ml. of 60 per cent perchloric acid. Do not add the perchloric acid before the nitric acid treatment because an explosion may occur. Evaporate to near dryness. Transfer residue quantitatively into a 125 ml. platinum dish, washing four or five times with 5 ml. portions of water. Add 5 to 8 ml. of 48 per cent hydrofluoric acid, from an 8 ml. beaker coated with paraffin, to the platinum dish; heat in hot plate carefully to dryness until silicon fluoride fumes are totally driven off. While working with grass samples from the dry area of Puerto Rico, a pink color persisted in this stage. It was destroyed by adding a pinch of peroxydisulfate ($K_2S_2O_8$) salt and a few drops of concentrated nitric acid. Cool, add 10 ml. of hot 0.6 normal hydrochloric acid, and dissolve the salts by continued heating and crushing of solid material with a flat end glass rod. Transfer to a 100 ml.

volumetric flask. Repeat heating-crushing operation, until the salts go in solution. Make up to 100 ml. volume with water and label, "Solution A"; 1 milliliter of this solution is equivalent to 0.075 gram of plant tissue.

Manganese. Manganese was determined by the simplified periodate method described by Peech (3). Pipette a 10 ml. aliquot of "Solution A" equivalent to 0.75 gram of plant tissue, into a 50 ml. beaker and evaporate to dryness, in a hot plate, to remove excess of hydrochloric acid. Dissolve residue in 6 ml. of normal nitric acid and transfer to a test-tube graduated at 11 ml. and follow the procedure explained before for the soils.

The transmittance-concentration curve (figure 3) was also developed as explained for the soils.

The amount of manganese in plant is calculated as follows:

$$\text{p.p.m. Mn in plant} = \frac{\text{milligrams Mn in curve} \times 1,000,000}{1000 \times 0.75}$$

$$= 1333 \times \text{mg. Mn in curve.}$$

Iron. Pipette 1 ml. of "Solution A" equivalent to 0.075 gram of plant tissue into a test-tube graduated to 10 ml. and develop color as explained before for soils.

Transmittance was measured this time in a Coleman spectrophotometer, model 11. The transmittance-concentration curve for iron (figure 7) was determined in the same standard used for soils. The following transmittances were obtained with filter PC-4, at a wave length of 490 mμ, using a reagent blank as reference solution:

STANDARD IRON SOLUTION		TRANSMITTANCES
ml.	*mg. Fe*	*%*
0.5	0.005	79.2
1.0	0.010	61.3
1.5	0.015	48.1
2.0	0.020	37.4
2.5	0.025	30.0
3.0	0.030	24.0

The amount of iron in plant is calculated as follows:

$$\text{p.p.m. Fe in plant} = \frac{\text{milligrams Fe in curve} \times 1,000,000}{1,000 \times 0.075}$$

$$= 13,333 \times \text{mg. Fe in curve.}$$

Phosphorus. Pipette a 0.1 ml. of solution A, equivalent to 0.0075 gram of plant tissue into a test-tube graduated at 10 ml. using a 0.1 ml.
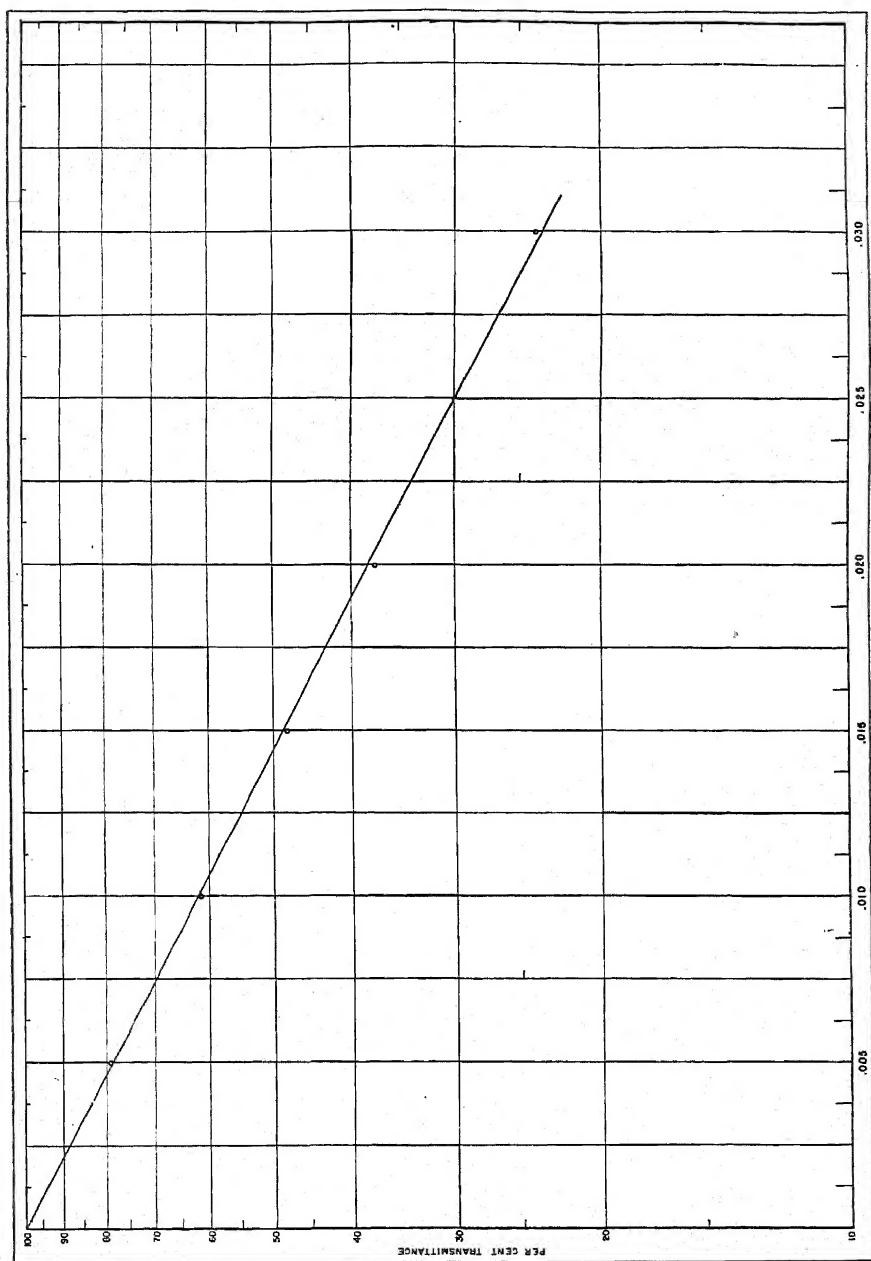
FIG. 7   Iron in plants.   Abscissa represents milligrams of iron as Fe.

Mohr's pipette.   Add 1 ml. of water and discharge the pipette by blowing with the tip under the water.   Add 1 ml. of ammonium molybdate solution (2.5 per cent in 5 normal sulfuric acid), mix, and add 0.4 ml. of 0.25 per cent aminonaphtholsulfonic acid solution (0.125 gram of aminonaphtholsulfonic acid to 49 ml. of filtered 15 per cent sodium bisulfite, and then adding 1.25 ml. of 20 per cent sodium sulfite).   Make to volume and mix.   Read transmittance in spectrophotometer.

The transmittance-concentration curve for phosphorus (figure 8) was obtained in a Coleman spectrophotometer, model 11, with filter PC-4, at a wave length of 600 mμ, using distilled water as reference solution.   The following transmittances were obtained in eight phosphorus standard solutions prepared as explained in the soils procedure:

STANDARD PHOSPHORUS SOLUTION		TRANSMITTANCE
ml.	mg. P	%
1.0	0.0050	83.1
1.5	0.0075	78.5
2.0	0.0100	72.9
3.0	0.0150	63.0
3.5	0.0175	58.6
4.0*	0.0200	54.1
5.0	0.0250	46.7
5.5	0.0275	43.5

The slope of this curve was found not to be constant.   It is suggested to run three standard solutions with the unknown.

The calculation of phosphorus in plant is as follows:

$$\text{p.p.m. P in Plant} = \frac{\text{milligrams P in curve} \times 1{,}000{,}000}{1{,}000 \times 0.0075}$$

$$= 133{,}333 \times \text{mg. P in curve.}$$

Removal of Iron, Aluminum, and Phosphorus Previous to Calcium and Magnesium Determinations.   Transfer a 2.0 ml. aliquot of solution A, equivalent to 0.15 gram of plant tissue, to a 15 ml. centrifuge tube graduated at 13 ml.   Add 0.2 ml. of ferric chloride solution (1.22 grams of ferric chloride hexahydrate in 250 ml. of 1 to 250 hydrochloric acid), mix, add 8 ml. of buffer solution (25 grams of sodium acetate, 62.5 grams of ammonium chloride, and 0.5 gram of sodium hydroxide in 1 liter of solution), and mix again.   Add 1 drop of methyl red indicator solution (0.02 per cent) and 0.6 N ammonium hydroxide until the color of the solution changes from slightly red to deep yellow, and then add 2 drops in excess.   Dilute to about 13.2 ml., mix with a stirring rod, and digest in a water bath at 80°C. for 5 minutes to flocculate the precipitate.   Mix thoroughly, and centrifuge
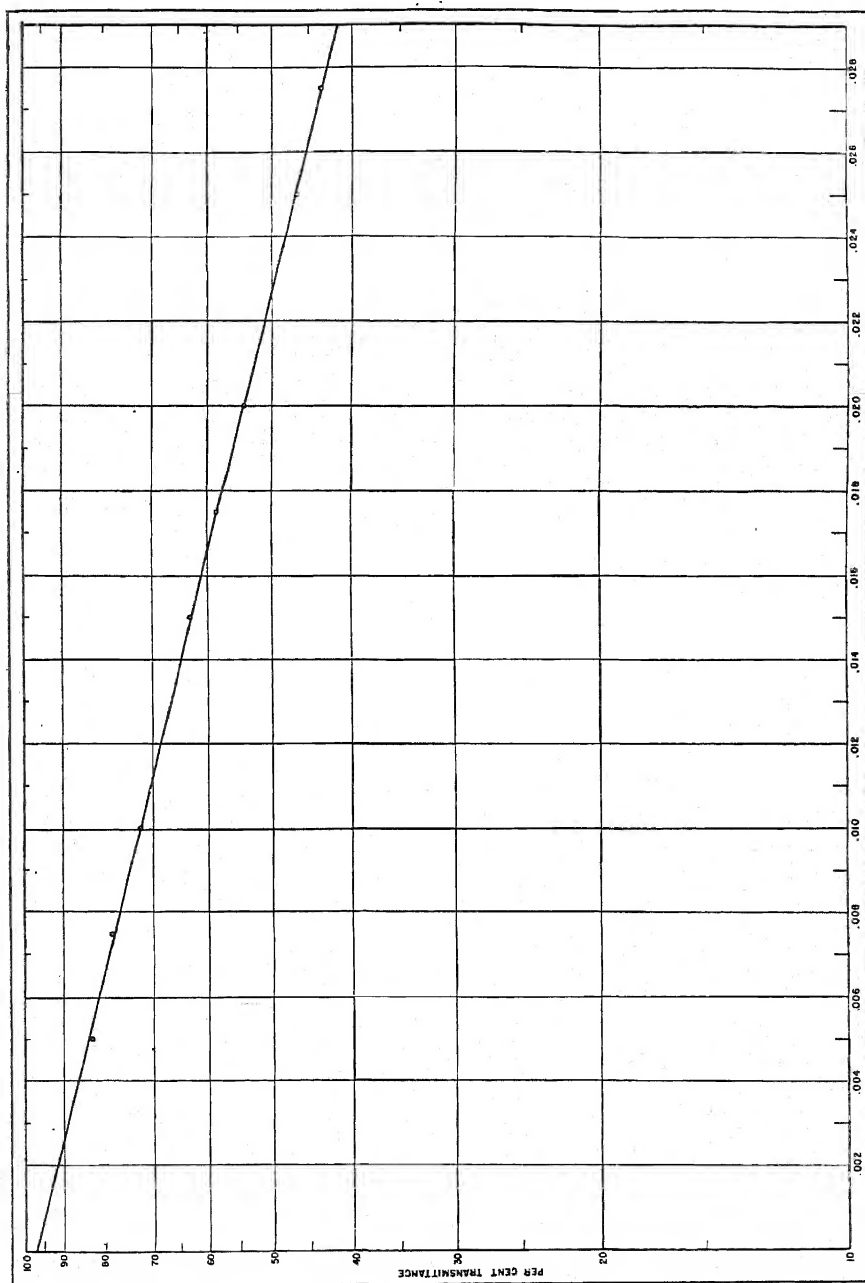
FIG. 8   Phosphorus in plants.   Abscissa represents milligrams of phosphorus as P.

while hot for 10 minutes.   The solution should have evaporated to 13.0 ml.

Calcium.   Calcium was determined by the method of Peech (3).   Transfer a 10 ml. aliquot from the above 13 ml. clear solution, equivalent to 0.1154 gram of plant tissue, into another calibrated 15 ml. centrifuge tube; add 1.4 ml. of 0.2 normal hydrochloric acid, and place in a water bath at 70°C.   Mix; add 2 ml. of 3 per cent ammonium oxalate, mix thoroughly again, and digest for 30 minutes at 70°C.   Remove the tube from the bath and let stand for 30 minutes.   Centrifuge for 15 minutes, at about 2000 r.p.m.   The volume should now be 13 ml.   Decant the clear supernatant liquid gently into a 25 ml. test-tube and save for the magnesium determination.

Allow the centrifuge tube to drain for several minutes, inclined at a 45° angle, on a filter paper.   Add quickly from a pipette, about 5 ml. of 2 normal ammonium hydroxide saturated with calcium oxalate, centrifuge for 15 minutes, decant carefully and discard the solution.   Drain the tube and save the precipitate.   One washing is sufficient unless very large quantities of calcium are present.   Add about 5 ml. of 10 per cent sulfuric acid, heat to 70°C. on a water bath, and titrate with standard 0.025 normal potassium permanganate.

$$\text{p.p.m. Ca in plant} = \frac{\text{ml. KMnO}_4 \times 0.025 \times 0.02004 \times 1,000,000}{0.1154}$$

$$= 43,413 \times \text{ml. KMnO}_4$$

Magnesium.   Magnesium was also determined by the method of Peech (3).   Pipette 10 ml. of supernatant liquid, from the solution set aside for the magnesium determination, equivalent to 0.0887 gram of plant tissue, in a 15 ml. centrifuge tube graduated at 13 ml. and proceed as described in the magnesium determination reported before for soils.   Take a 2 ml. aliquot from the 13 ml. solution, equivalent to 0.01365 gram of plant tissue, and develop color as mentioned previously for soils.   Read transmittance in curve (figure 1).

$$\text{p.p.m. Mg in plant} = \frac{\text{milligrams Mg in Curve} \times 1,000,000}{1,000 \times 0.01365}$$

$$= 73,260 \times \text{mg. Mg in Curve}$$

Proteins, Ether Extract and Fiber.   Proteins, ether extract, and fiber were determined in the first, second and third crops.   Proteins were also determined in the fourth crop, previous to and after the second application of ammonium sulphate.

PRESENTATION AND DISCUSSION OF DATA OBTAINED

The mineral changes brought about in the soil, fifteen and twenty-three months after the lime application, are expressed in table 2.

The increase of available calcium and phosphorus and the decrease of available iron in the soil due to liming, was highly significant, fifteen and twenty-three months after the lime was applied to the soil.   The decrease of available manganese in the soil due to liming was highly significant fifteen months after liming and significant twenty-three months after liming.   The difference between the available magnesium content of the limed and unlimed soil was not significant.

TABLE 2

Parts per million of available calcium, magnesium, manganese, phosphorus, and iron in soil type Fajardo clay, unlimed and limed (dry basis)

TIME OF SAMPLING	TREATMENT	CALCIUM (Ca)	MAGNESIUM (Mg)	MANGANESE (Mn)	PHOSPHORUS (P)	IRON (Fe)
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
15 months after liming	Unlimed	849	180	42	13	17
	Limed	6831	172	8	61	2
23 months after liming	Unlimed	992	156	29	21	45
	Limed	5351	156	5	56	12

TABLE 3

Parts per million of calcium, magnesium, manganese, phosphorus and iron in three crops of Para-Carib grass grown in soil Type Fajardo Clay, unlimed and limed (air dry basis)

CROP NUMBER	TIME OF SAMPLING	TREATMENT	CALCIUM (Ca)	MAGNESIUM (Mg)	MANGANESE (Mn)	PHOSPHORUS (P)	IRON (Fe)
			p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Second	14 months after liming	Unlimed	2199	1509	229	2100	149
		Limed	2811	1638	137	2430	158
Third	17 months after liming	Unlimed	2008	1824	156	2749	196
		Limed	3351	2212	84	3047	160
Fourth	32 months after liming	Unlimed	2919	2166	243	2450	124
		Limed	3381	2088	181	2929	121

The mineral changes brought about in the grass after the lime application are expressed in table 3.

The increase of calcium and the decrease of manganese in the grass due to liming was highly significant for the second and third crops while the increase of calcium was significant for the fourth crop, and the decrease of manganese was not significant.   The increase of phosphorus in the grass due to liming was highly significant for the second and fourth crops but was not significant for the third crop.   There was no significant change in the iron

content of the grass due to liming in the three crops and in the magnesium content of the second and fourth crops. However, the increase of magnesium in the grass crop due to liming was highly significant for the third crop.

The average total yield of green grass per acre in the unlimed and limed soil for each of the first five consecutive crops, and for the five crops, is reported in table 4.

The increase in the grass yield due to liming was significant for the first and third crops. However, the difference between the respective yields of the unlimed and limed soil for the second, fourth and fifth crops, and for the total of five crops, was not significant.

TABLE 4

Yield in tons per acre of green Para-Carib grass in Fajardo clay unlimed and limed

| TREATMENT | NUMBER OF CROP | | | | | TOTAL |
	1 No nitrogen applied	2 No nitrogen applied	3 Nitrogen applied	4 No nitrogen applied	5 Nitrogen applied	
Unlimed...............	8.98	7.47	9.59	8.92	9.82	44.78
Limed.................	11.00	8.03	10.33	8.62	9.81	47.79

TABLE 5

Grass yields of table 4 expressed as tons per acre per month of green grass

| TREATMENT | NUMBER OF CROPS AND AGE IN MONTHS | | | | |
	1 5.75 mo.	2 8.25 mo.	3 3.50 mo.	4 4.25 mo.	5 3.50 mo.
Unlimed..........	1.56	.91	2.74	2.10	2.81
Limed............	1.91	.97	2.95	2.03	2.80

The monthly rate of growth for each of the five grass crops is reported in table 5. The age of the crop used for this calculation was the mean of that reported in table 1.

The increase in the yield of the third grass crop is due to nitrogen fertilization. It gave about two tons of green grass more per acre than the previous crop (table 4). The monthly rate of growth was about three times higher (table 5). In a period of 7.75 months, the third and fourth crops combined gave close to 5 tons of green grass per acre, while the unfertilized second crop in 8.25 months gave about one ton. In fourteen months the first two unfertilized crops gave about three tons of green grass per acre.

However, in about a period of one year the last three crops gave about eight tons of green grass per acre. The eight-ton year yield was obtained with two applications of nitrogen fertilizer, one to the third crop and another to the fifth crop, each at the rate of 500 pounds of ammonium sulphate per acre.

The increase of grass yield is not the only advantage obtained when nitrogen is applied. The content of the nitrogen in the crop is also increased if the grass is cut early (table 6).

The protein content of the Para-Carib grass mixture ranged between 3 and 4 per cent. Grass from the third crop taken 36 days after the first nitrogen application gave around 11 per cent protein or about three times that in the original grass. The ammoniacal content in the third crop was .07 and .05 per cent, respectively, for the unlimed and limed grass. The protein content of the fifth grass crop, collected 82 days after the nitrogen application, was about 5 per cent.

TABLE 6

Protein content of Para-Carib grass in five consecutive crops, before and after nitrogen fertilization (air-dried-basis)

TREATMENT	CROP 1, NO NITROGEN APPLIED	CROP 2, NO NITROGEN APPLIED	CROP 3, 36 DAYS AFTER FIRST NITROGEN APPLICATION	CROP 4, 180 DAYS AFTER FIRST NITROGEN APPLICATION	CROP 5, 82 DAYS AFTER SECOND NITROGEN APPLICATION
	%	%	%	%	%
Unlimed.........	3.6	3.8	11.7	3.3	4.8
Limed...........	3.8	3.7	10.8	3.6	4.5

SUMMARY

This paper reports the procedures followed for the chemical determinations of exchangeable calcium, magnesium, and manganese; and available phosphorus and iron in soils; and for the total amount in plants of each of those minerals mentioned. Spectrophotometric methods are given for magnesium and manganese in soils and plants; and for phosphorus and iron in plants including the transmittance-concentration and spectral-transmittance curves for each of these elements. Photocolorimetric methods are also given for available iron and phosphorus in soils with their corresponding curves.

This paper reports also changes of the minerals calcium, magnesium, manganese, phosphorus and iron in an acid soil, 15 and 23 months after liming. It also reports changes of these minerals in each of five crops of a mixture of Para grass *Panicum purpurascens*, and Carib grass *Eriochloa polystachya*, grown in the unlimed and limed soil. The yield of green grass is also reported for each crop.

The important results are as follows:

1. Significant increases of available calcium and phosphorus and significant decreases of available manganese and iron in the soil, due to liming, and no significant difference of the available magnesium content.

2. Significant increase of calcium and significant decrease of iron in each of three consecutive crops of the grass, due to liming. Significant decrease of manganese in the first two crops but no significant difference in the third crop. No significant difference in the magnesium content of the first and third crops but a significant difference in the middle crop.

3. The increase in the grass yield, due to liming, was significant for the first and third crops but was not significant for the second, fourth, and fifth crops, or for the total of the five consecutive crops.

4. An application of 500 pounds of ammonium sulphate per acre gave about two tons of green grass more per acre than a previous unfertilized crop. The period of growth of the fertilized grass was 3.5 months while that of the unfertilized grass was 8.25 months.

5. Grass collected early, 36 days after the nitrogen application, contained around 11 per cent of protein or about three times as much as in the unfertilized grass.

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TRACING THE MINERAL FROM THE SOIL TO THE PLANT TO THE ANIMAL BLOOD

PART II. EFFECT OF UNLIMED AND LIMED GRASS ON THE CHEMICAL COMPOSITION OF GOATS' BLOOD

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The effect of lime applications on the composition of calcium, magnesium, manganese, phosphorus and iron, in the acid red soil type "Fajardo clay", and on a mixture of "Para and Carib" grass, was discussed in Part I published by Bonnet and Riera (1).

This paper presents information on the effect of the unlimed grass, the limed grass, and the limed grass supplemented with manganese per os, on the weight of the goat; on the grass consumed; and on the hemoglobin, calcium, phosphorus, iron, hematocrits, red-blood cells, and white-blood cells of the goats' blood.

EXPERIMENTAL

The layout of the experimental field was explained in Part I. All the grass cut daily from the strips of the unlimed plots was chopped into small pieces and mixed into a sample labeled "Unlimed grass". An identical sample from the limed plots was labeled "Limed grass".

Fifteen one-year virgin female goats were selected for the experiment. They were given the parasite treatment: twelve grams of phenothiazine per os. The animals were randomized, one for each of fifteen pens (see photo), into five groups for the following three treatments: 1) goats fed with unlimed grass, 2) goats fed with limed grass, 3) goats fed with limed grass, and in addition, fed per os, with manganese sulphate.

The goat experiment covered an eleven-month period. It was started on October 19, 1944 and finished on September 15, 1945. The experiment was divided into four periods as follows:

1. Pre-feeding period (October 19–November 14, 1944)
2. Pre-gestation period (Nov. 15, 1944–January 15, 1945)
3. Gestation period (January 16–July 15, 1945)
4. Lactation period (July 16–September 15, 1945)

Eight pounds of the chopped "Unlimed grass" were fed to each of the goats in treatment No. 1. Eight pounds of the chopped "Limed grass" were fed to each of the goats in treatments No. 2 and No. 3. The feeding box (see photo) in each pen avoided the contamination of the grass with urine and excrement.

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Each animal in treatment No. 3 was supplied daily in addition, per os, a solution containing 0.453 gram of manganese sulphate ($MnSO_4H_2O$) per liter, equivalent to .147 milligrams of manganese per milliliter. From November 15 to November 29, 1944, inclusive, and from February 3, 1945 to July 15, 1945, inclusive, each goat received daily, per os, 1 ml. of the manganese solution, but for the period between November 30, 1944 to February 2, 1945, inclusive, each goat received daily, per os, 1.5 ml. of the manganese solution. For the eight-month period, covering pre-gestation and gestation, each goat received 275.5 milliliters of the manganese solution equivalent to 40.5 milligrams of manganese.

The amount of residual grass left daily by each animal was also weighed. A composite sample from the "Unlimed Grass" and the "Limed Grass", was taken daily for moisture analysis. A record was kept of the amount of green and dry grass consumed by each animal.

Rain water from the two concrete wells besides greenhouse No. 5 in the Experiment Station Farm, was supplied daily to each animal. The water consumed daily, however, was very low. The mineral content of this water was as follows:

	parts per million
Calcium	4.0
Phosphorus	2.0
Iron	0.03
Magnesium	None
Manganese	None

Each goat was weighed three times in three consecutive days, around the middle and the end of each month.

To induce breeding of the goats at approximately the same time, each animal was given, per os, on January 29, 1945, 5 milligrams of diethyl stylbestrol.

METHODS OF ANALYSES

Blood samples were taken from each animal at the beginning of the "Pre-Feeding Period" on October 19, 1944; one month after the beginning of the "Pre-Gestation Period" in the middle of December 1945; and thereafter every middle of the month up to August 1945. About 10 ml. of blood were drawn from each animal by a direct puncture of the jugular vein: 2 ml. for the hematological test and 8 ml. for the chemical test. The 2 ml. blood portion was poured into a 10 ml. test tube containing a dry oxalate salt. This salt was prepared by adding 0.1 ml. of a mixed solution of 6 per cent ammonium oxalate and 4 per cent of potassium oxalate to each tube, and evaporating to dryness.

Hematological Test

A 0.1 ml. of the oxalated blood was used for the red-blood cell and white-blood cell counts. A 0.7 ml. portion of this blood was used for hematocrits and 0.1 ml. for hemoglobin.

Hemoglobin

Hemoglobin was determined in a fresh sample of cow's blood by the Van Slyke's method (2). Its content was found to be 9.04 grams hemoglobin per 100 ml. blood. A 1:25 solution was prepared by diluting 2 ml. of this cow's blood to 50 ml. with 0.1 per cent sodium carbonate solution. Transmittances of eleven dilute solutions, prepared from the 1:25 blood solution, are as follows:

SOLUTION NO.	ALIQUOT 1:25 BLOOD SOL	DILUTED WITH 0.1% SODIUM CARBONATE TO	DILUTION RATIO	HEMOGLOBIN MILLIGRAMS PER ML. BLOOD	TRANSMITTANCE
	ml.			mg.	%
1	6.00	18.00	1: 75.0	1.2053	15.0
2	5.00	20.00	1: 100.0	0.9040	22.5
3	5.00	23.00	1: 114.7	0.7881	26.7
4	5.00	26.67	1: 133.3	0.6782	31.3
5	5.00	32.50	1: 162.5	0.5563	38.1
6	2.50	20.00	1: 200.0	0.4520	45.1
7	2.50	26.67	1: 266.7	0.3390	54.8
8	1.25	20.00	1: 400.0	0.2260	66.8
9	1.25	26.67	1: 533.4	0.1695	73.6
10	1.25	40.00	1: 800.0	0.1130	81.0
11	1.25	80.00	1:1600.0	0.0565	90.0

The transmittance was determined in a Coleman spectrophotometer, model 11, using a PC-4 filter at a wave length of 540 mμ where maximum color absorption (figure 2) occurs. The sodium carbonate solution was used as reference. It gave a 99.1 per cent transmittance when the instrument was set at zero. The transmittance-concentration curve obtained for hemoglobin is reported in figure 1.

For the determination of hemoglobin in the unknown, 0.1 ml. of oxalated blood was diluted to 20 ml. with 0.1 per cent sodium carbonate solution. The transmittance of the colored solution was read in a Coleman spectrophotometer, model 11, using filter PC-4 at a wave length of 540 mμ. The transmittance from the curve (figure 1) multiplied by 20 gives grams of hemoglobin per 100 ml. blood.

FIG. 1. Hemoglobin in blood. Curve obtained in Coleman spectrophotometer, Model 11, with filter PC-4, at a wave length of 540 mu, and 0.1 per cent sodium carbonate as reference solution. Abscissa represents milligrams of hemoglobin per milliliter blood. On the basis of 0.1 ml. blood diluted to 20 ml. with 0.1% NaCO₃; value in curve × 20 = grams hemoglobin per 100 ml. blood.

Chemical test

Iron.   A sample of 0.5 ml. of oxalated blood was taken for the iron determination.   The Wong's (5) modified method was used to develop the color and the transmittance was read in the Coleman spectrophometer with filter PC-4 at a wave length of 480 mμ using a reagent blank as the reference solution.   The method used was as follows: Transfer with an Ostwald pipette 0.5 ml. of blood into a 50 ml. volumetric flask and introduce 2 ml. of iron-free concentrated sulphuric acid.   Whirl the flask to agitate the mixture for 1 or 2 minutes.   Add 2 ml. of saturated potassium persulfate solution and
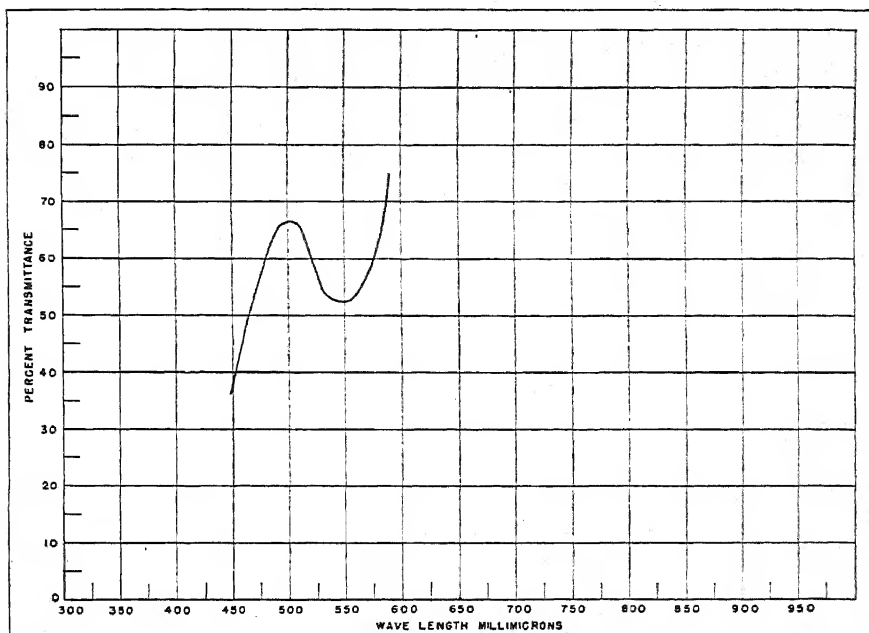
FIG. 2. Spectral-transmittance curve for hemoglobin.   Maximum absorption of light at 540 millimicrons.

shake.   Dilute to about 25 ml. with distilled water and add 2 ml. of 10 per cent sodium tungstate solution.   Mix, cool to room temperature under the tap and then dilute to volume with distilled water.   Stopper the flask and invert two or three times to effect thorough mixing.   Filter through a dry filter paper into a clean, dry receiving vessel.   Pipette 20 ml. of the clear filtrate into a large test-tube graduated at 20 ml. and 25 ml.   The color was developed by adding 1 ml. of saturated potassium persulfate solution and 4 ml. of 3 N potassium sulfocyanate, KCNS, solution.

The iron standard solution was prepared as follows: Transfer 0.8635

gram of crystallyzed ferrous ammonium sulphate, $FeNH_4(SO_4)_2 \cdot 12H_2O$, to a small beaker and dissolve in about 50 ml. of water. Add 20 ml. of 10 per cent iron-free sulphuric acid. Transfer quantitatively to a liter volumetric flask and dilute to the liter mark with water. One ml. of this solution contains 0.1 mg. Fe. Dilute 10 ml. of this standard solution to 100 ml. with distilled water. One ml. contains 0.01 mg. Fe. The equivalent amount of this standard iron solution was measured in a pipette and poured into the 25 ml. test tube; 0.8 ml. of iron free concentrated sulphuric acid was added, and diluted to the 20 ml. mark with distilled water. Cool to room temperature under the tap, and develop the color as mentioned above, but develop it at the time of reading in the spectrophotometer to avoid fading. The transmittance obtained, for the various iron concentrations using a PC-4 filter, and a wave length of 480 mμ in the Coleman spectrophotometer, model 11, using the reagent blank as reference solution, was as follows:

IRON CONCENTRATION	TRANSMITTANCE
mg.	%
0.01	83.5
0.02	71.2
0.03	60.0
0.05	42.7
0.07	30.6
0.08	26.0
0.10	19.0

The slope of the standard curve (figure 3) remains constant. Readings from curve give milligrams Fe per 100 ml. blood. The lower transmittance or maximum light absorption was obtained at a wave length of 480 mμ (figure 4).

Calcium and Phosphorus. The non-oxalated blood was centrifuged, immediately after drawn to avoid hemolysis, for 5 minutes at 2800 r.p.m. in an International clinical centrifuge. The fibrin sealing the plasma was loosened carefully with a wooden rod. The plasma was poured down, or was centrifuged again if necessary, to avoid hemolysis since the red-blood cells of goat's blood are quite minute (diameter = 4.1 microns) and fragile.

Calcium. Calcium in blood serum was determined by the method of Roe and Kahn (4) using a Klett-Summerson photoelectric colorimeter No. 2141, test-tube model, with red filter 66, at a wave length range 640–700 mμ, reading against a reagent blank. The procedure for the blank, unknown, and standard, was as follows:

Unknown. Add 1 volume of serum to 4 volumes of 10% trichloroacetic acid in a small flask and shake well. Pour onto a dry calcium-free filter
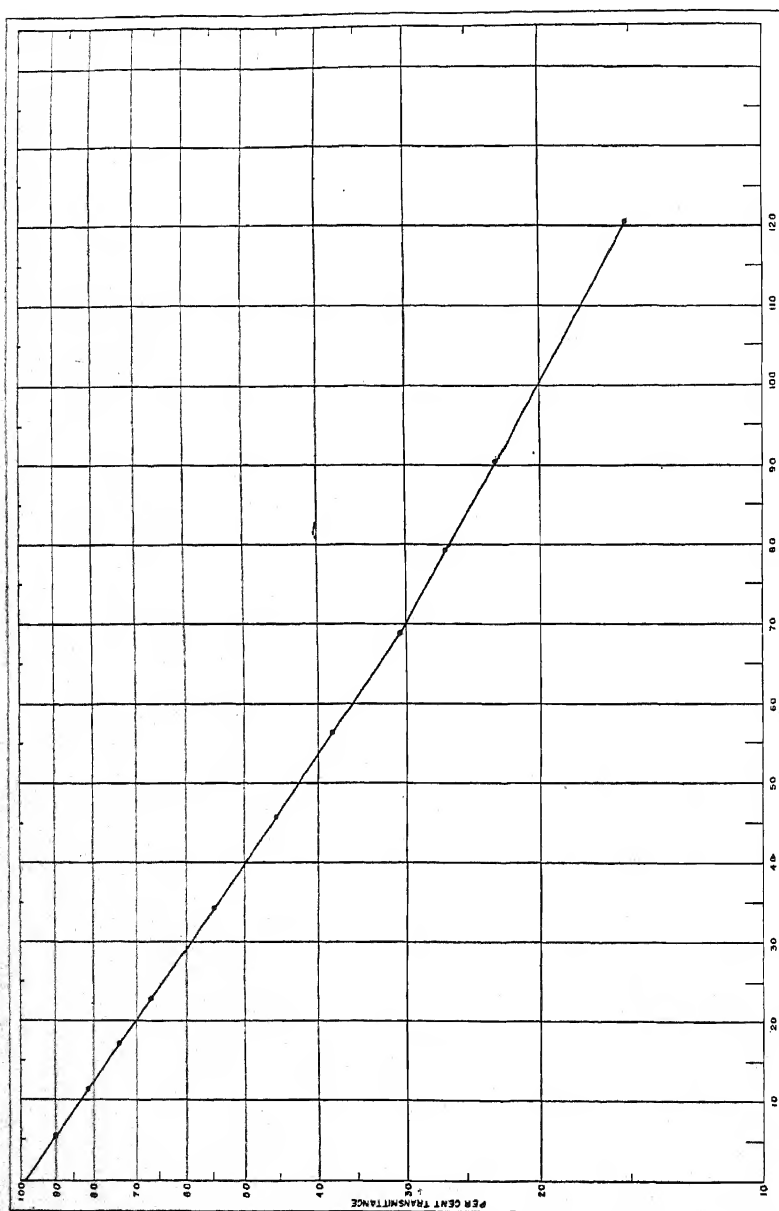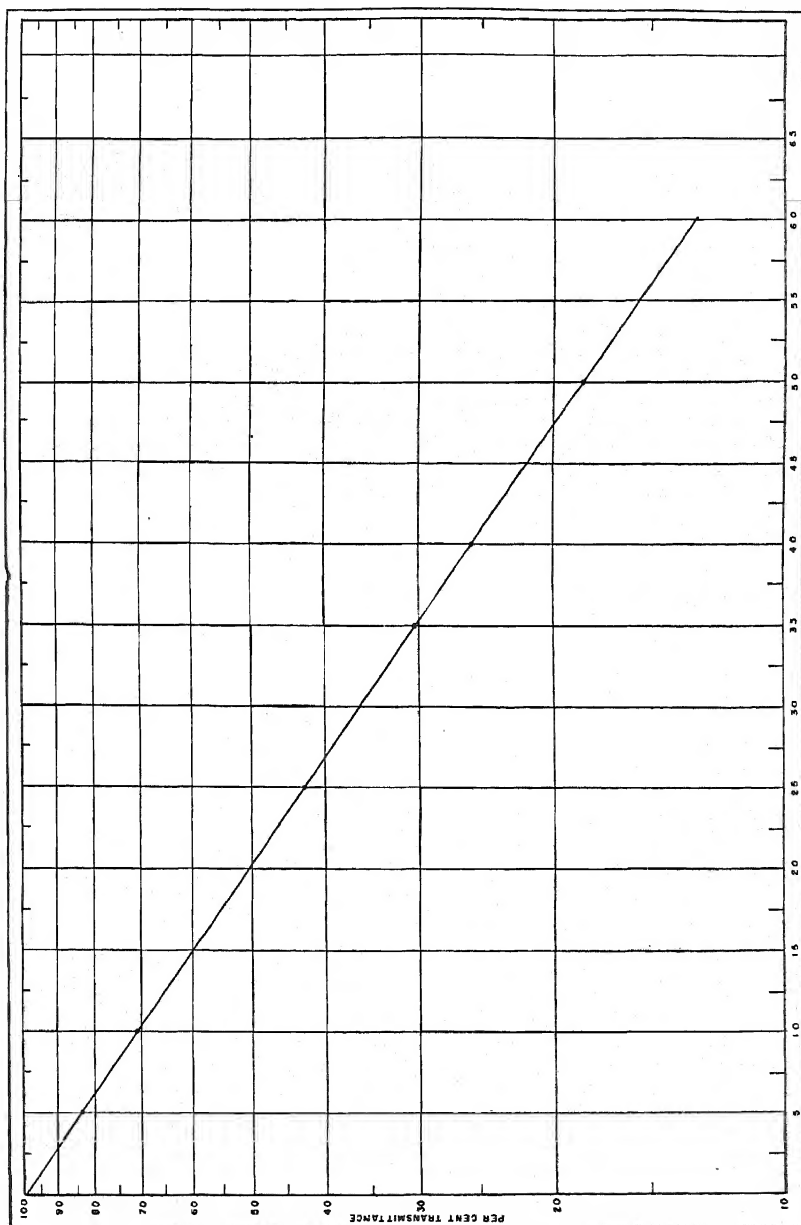
FIG. 3. Iron in blood curve obtained in Coleman spectrophotometer, Model 11, with filter PC-4, at a wave length of 480 mu, and reagent blank as reference solution. Abscissa represents milligrams of iron as Fe. Readings from curve give milligrams Fe per 100 ml. blood.

paper (Whatman No. 42 or its equivalent) and collect the filtrate in a dry flask. Place 5.0 ml. of the filtrate in a graduated 15 ml. conical centrifuge tube and add 1.0 ml. of 25% sodium hydroxide solution, with mixing by lateral shaking. Allow to stand for 5 minutes, then add 1.0 ml. of 5% trisodium phosphate solution, mix well by lateral shaking, and set aside for an hour. At the end of this time, centrifuge for 2 minutes, and pour off the supernatant fluid, allowing the tube to drain in an inverted position for 2 minutes. Wipe the mouth of the tube dry with a clean cloth. Wash the
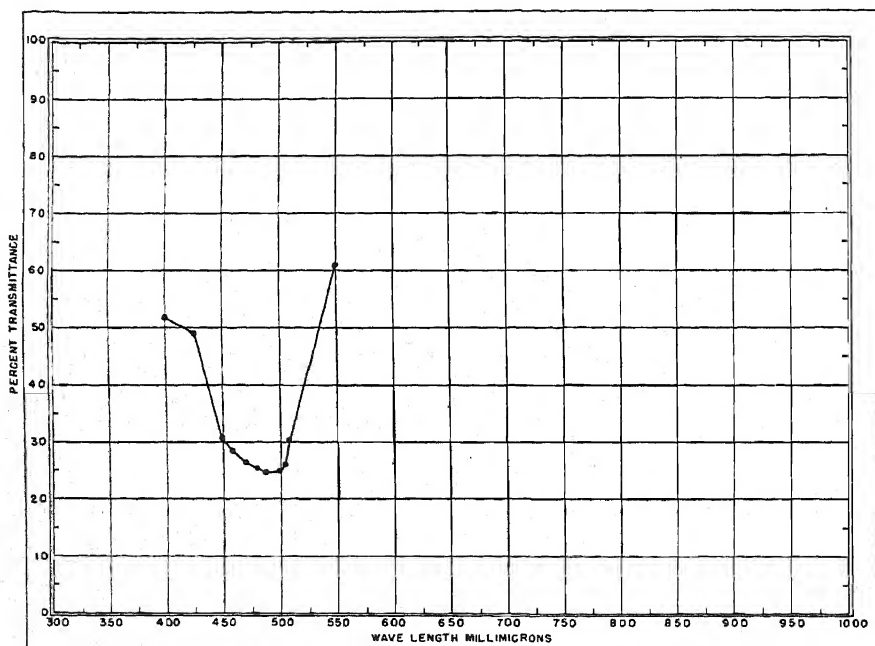
Fig. 4. Spectral-transmittance curve for iron as per method of blood. Maximum absorption of light at a wave length of 480 millimicrons.

precipitate with 5 ml. of alkaline-alcoholic wash reagent (to 10 ml. of amyl alcohol, add 58 ml. of ethyl alcohol, and mix; dilute to 100 ml. with water; add 2 drops of 1 per cent phenolphthalein solution and then, drop by drop, add sufficient 5 per cent sodium hydroxide solution to a distinct pink color; (a few drops should be sufficient); delivered from a pipette with a fine tip, blowing the first portion of wash fluid against the precipitate with such force as to break it up, and using the remainder of wash fluid to rinse down the sides of the centrifuge tube. If necessary use a stirring rod to break up the precipitate. Centrifuge for 2 minutes, pour off the supernatant fluid and allow the tube to drain as before. After draining, wipe the mouth of the

tube dry, and add 2.0 ml. of molybdate reagent (dissolve 25 grams of c.p. ammonium molybdate in 200 ml. water, and pour into a one-liter volumetric flask containing 500 ml. of 10 normal sulfuric acid; dilute to the mark and mix); to dissolve the precipitate and form phosphomolybdate from the phosphate present. After a complete solution of the precipitate, which may be hastened by shaking or stirring, dilute to 10.0 ml. with distilled water, and mix well. Transfer a 5.0 ml. portion from the centrifuge tube to a colorimeter tube and add 0.4 ml. of the aminonaphtholsulfonic acid reagent. Dilute with water to 10.0 ml., mix, and read in the colorimeter after five minutes setting the colorimeter at zero with reagent blank.

Blank. Treat a 5.0 ml. portion of distilled water with 1 ml. of molybdate reagent and 0.4 ml. of aminonaphtholsulfonic acid reagent (weigh 0.125 gram of c.p. 1-amino-2-naphthol-4-sulfonic acid, Eastman Kodak 360, into a 250 ml. beaker containing 50 ml. of 15 per cent sodium bisulfite and 1 ml. of 20 per cent sodium sulfite; shake until dissolved; add a little more of the sodium sulfite solution if necessary to bring the powder into solution; add a little more of the sodium sulfite solution, but an excess should be avoided; keep in a brown bottle away from light; this solution should be prepared fresh every two weeks); and dilute to the 10.0 ml. mark. Set the colorimeter at zero with this reagent blank.

Standard. Treat a 5.0 ml. portion of the standard phosphate solution with 1 ml. of molybdate reagent, add 0.4 ml. of aminonaphtholsulfonic acid reagent, dilute with water to the 10.0 ml. mark, and mix. Read in the colorimeter after 5 minutes. 1 ml. standard phosphate solution = 0.001 milligram $P = \dfrac{9.67}{5}$ or 1.934 milligrams Ca per 100 ml. blood serum. The readings obtained for the standard curve, after setting instrument at zero with reagent blank, were as follows:

STANDARD P SOLUTION	PHOTOCOLORIMETER READINGS	Ca PER 100 ML. SERUM	SLOPE OF CURVE
mg.		*mg.*	
0.002	76	3.87	0.05092
0.004	155	7.74	0.04994
0.005	192	9.67	0.05036
0.006	228	11.60	0.05088
0.008	310	15.47	0.04990

The slope of the standard curve remains constant. Its average value or factor (figure 5) equals 0.05040. Reading of unknown in photocolorimeter × 0.05040 equals milligrams of calcium per 100 milliliters blood serum.

Inorganic Phosphorus. Inorganic phosphorus in blood serum was deter-

mined by the method explained in Levinson & MacFate (3) but using a
Coleman spectrophotometer, model 11, with filter PC-4 and a wave length
of 600 mμ (figure 7), reading against a reagent blank. The procedure was
as follows: Transfer 4 ml. of 10 per cent trichloracetic acid to a small flask
and add 1 ml. of the blood serum with shaking. Larger volumes of the
serum may be used in the same proportion. Shake well, let stand for 1 to 2
minutes, and filter through a phosphorus-free filter paper. If small quan-
tities of material are used, pour the mixture into a centrifuge tube, centri-
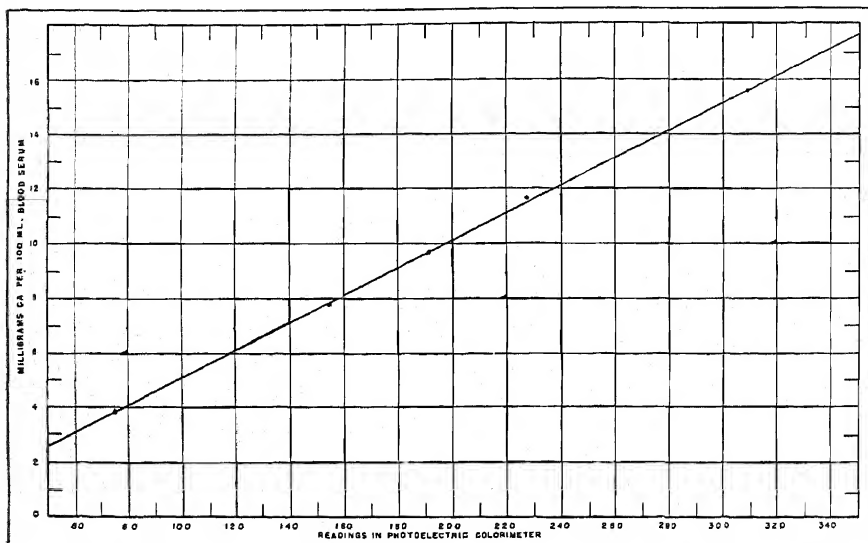
FIG. 5. Calcium in blood serum. Curve obtained in Klett-Summerson photoelec-
tric colorimeter, No. 2141, test tube model, with red filter 66, at a wave length range
640–700 mu, and the instrument at zero with reagent blank. Average slope of curve =
0.0504. Readings of curve ×0.0504 = milligrams of Ca per 100 ml. blood serum.

fuge for a few minutes and filter the clear supernatant fluid through
4.25 centimeter filter paper in a small funnel. Transfer 2 ml. of the filtrate
to a 50 ml. Erlenmeyer flask. Add 12 ml. of distilled water, 4 ml. of the
molybdic-sulfuric acid reagent A (50 ml. of 10 normal sulphuric acid added
to 50 ml. of 7.5 per cent sodium molybdate solution), and 2 ml. of dilute
stannous chloride solution (dissolve 10 grams of stannous chloride in 25 ml.
of concentrated hydrochloric acid; preserve this stock solution in a brown
bottle; dilute 1 ml. of stock solution to 200 ml. with water; preserve in a
brown bottle; this dilute solution keeps for 5 days, but is better if pre-
pared fresh each time). Mix well and after 15 minutes read in spectro-
photometer.
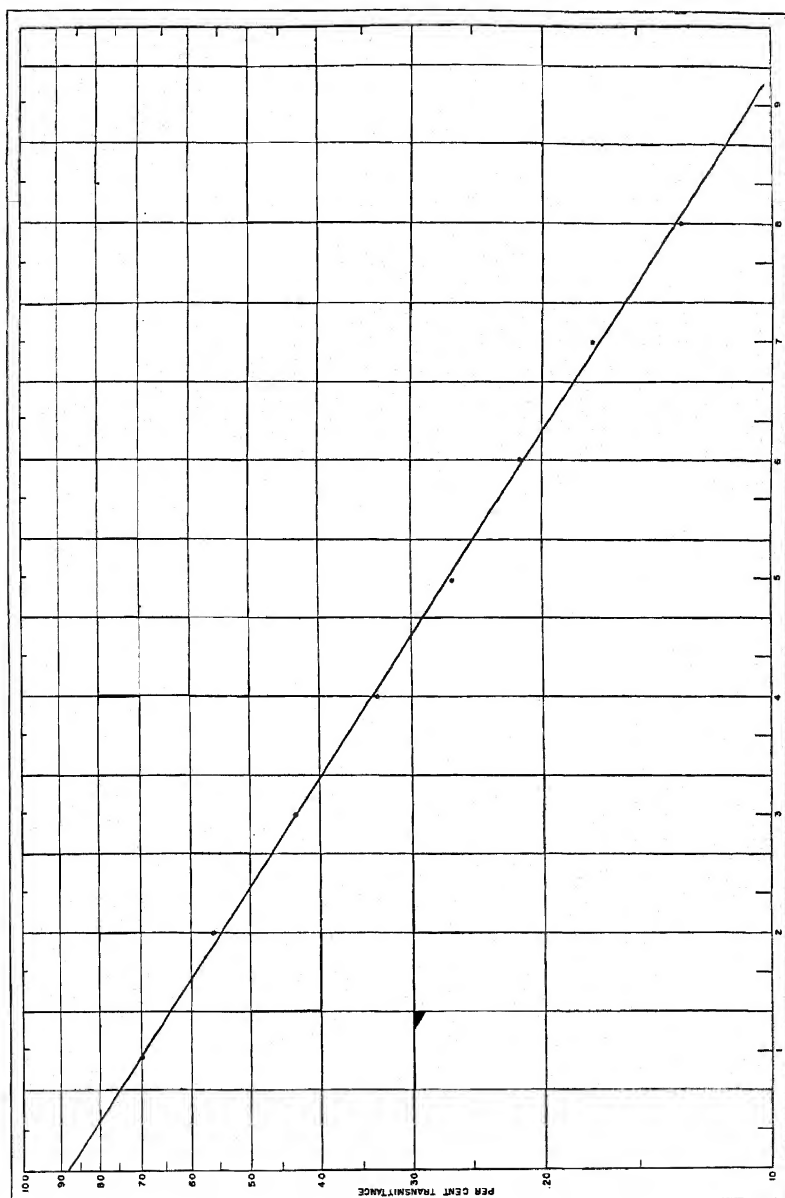
FIG. 6. Inorganic phosphorus in blood serum. Curve obtained in Coleman spectrophotometer, Model 11, with filter PC-4, at a wave length of 600 mu, and the reagent blank as reference solution. Abscissa represents milligrams phosphorus (P) per 100 ml. serum. Slope of standard curve was not found to be constant. Run at least three standards to check slope.

Calibration Curve.   Dilute 20 ml. of standard phosphorus solution (1 ml. = 0.01 mg. P) to 25 ml. with distilled water.   Dilute to 14 ml.   Add 4 ml.
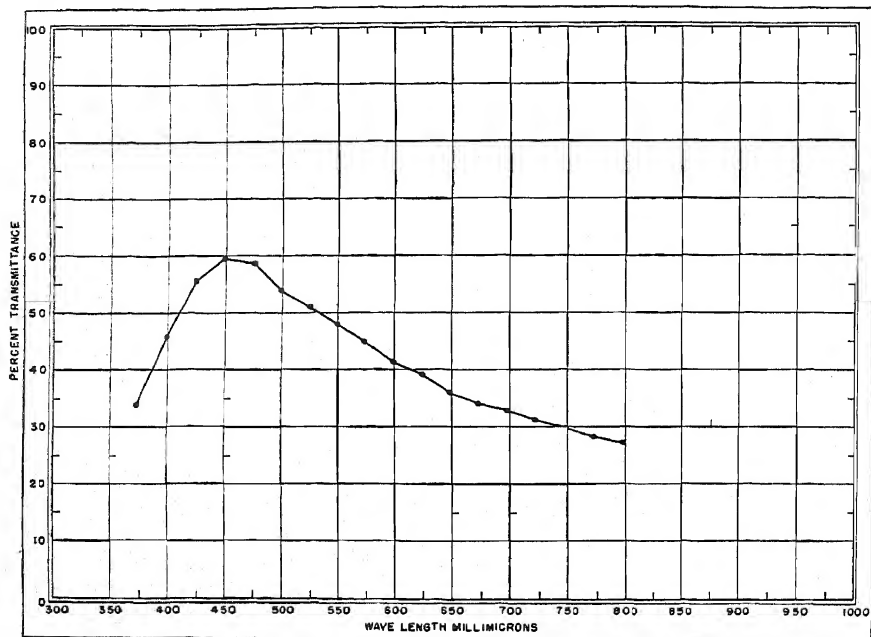
FIG. 7. Special-transmittance curve for phosphorus as per method of blood serum. The PC-4 filter was used for wave lengths, from 350 to 700 mu, and the PC-5 filter, from 700–800 mu.

of the molybdic-sulfuric acid reagent A and 2 ml. of the stannous chloride solution.   Read after 15 minutes and plot in semi-log paper.

The following readings were obtained for transmittance:

P-STANDARD	P IN STANDARD	P PER 100 ML. SERUM	TRANSMITTANCE
ml.	mg.	mg.	%
0.5	0.004	1	70.0
1.0	0.008	2	56.3
1.5	0.012	3	43.7
2.0	0.016	4	34.4
2.5	0.020	5	27.0
3.0	0.024	6	21.9
3.5	0.028	7	17.5
4.0	0.032	8	13.2

Prepare three sets of standards with 1, 5, 8 mgm. P per 100 ml. serum, respectively, simultaneously with the unknown, since it was found that the slope of the standard curve (figure 6) is not constant.

PRESENTATION OF DATA AND DISCUSSION

The monthly weights in pounds per goat and the average monthly weights for the five goats in the treatments: unlimed grass, limed grass, and limed grass plus manganese, are reported for a period of eleven months in table 1. The changes in average weight of the animals in each treatment before and after the pre-gestation period and after the gestation period are condensed in table 2. There was no significant increase in the weight of the goats for the two-month pre-gestation period. However, there was a higher significant decrease of about 8.5 pounds in weight after the six-month gestation period.

The total amount in pounds of the Para-Carib grass mixture and minerals, on the dry basis, eaten by each goat and the average eaten by the five goats in each of the three treatments during the pre-gestation and gestation periods are reported in table 3. The per cent of calcium, phosphorus, manganese, iron and magnesium used for the calculation is that reported previously (1) in part I for the third and fourth grass crops, respectively, in the field at the time of sampling. The total amount of dry grass eaten in the pre-gestation and gestation periods and the total minerals eaten for both periods by the five goats in each treatment are condensed in table 4.

There were significant differences for the pre-gestation period between the grass intake of the goats under the three treatments. The goats fed with unlimed grass ate less than those fed with limed grass. However, for the gestation period the difference for the grass intake between the treatments was not significant.

The average daily grass and mineral intake, in grams per five goats, of about 52 pounds in average weight, is reported in table 5. These data were calculated for a total of 243 days covering the pre-gestation and gestation periods.

The average intake of unlimed grass was about 6 pounds of dry grass per five goats per day and of limed grass was about 7 pounds for animals weighing around 52 pounds.

The goats fed with limed grass received daily about 4 grams more of calcium, 2 grams more of phosphorus, 0.2 grams more of iron, 1 gram more of magnesium, and about 0.2 grams less manganese than the goats fed with unlimed grass. The additional manganese added, per os, to each goat fed with the limed grass, which amounted to 41 milligrams of manganese for the 243 days covering the pre-gestation and gestation periods, was too small to account for an increase in the manganese content of the limed grass.

The average hemoglobin, iron in blood, calcium and phosphorus in blood serum, and blood count per goat in each treatment is reported in table 6. There were no significant differences in the pre-gestation period between the mean red or white-blood cells, the hematocrits, the hemoglobin and iron

TABLE 1

Monthly weights of goats fed with unlimed grass and limed grass with or without extra manganese, for a ten-month period

GROUP	TREATMENT	ANIMAL NUMBER	1944		1945								
			Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
			lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
I	Unlimed grass	81	57.2	56.7	54.8	53.5	51.3	49.5	46.2	48.8	D*	—	—
		90	69.5	68.0	68.8	64.0	64.5	60.2	59.3	54.2	57.7	60.3	58.2
		91	49.5	50.7	49.3	49.0	48.8	46.5	44.8	44.8	43.3	45.2	45.7
		92	48.0	50.0	47.8	45.2	42.7	39.8	37.0	40.0	38.8	39.8	40.3
		94	52.9	54.8	54.0	51.0	52.3	48.8	47.0	48.8	48.0	51.8	51.5
Ave............................			55.4	56.0	55.0	52.5	51.9	49.0	46.9	47.3	47.0	49.3	48.9
II	Limed grass	72	39.3	43.3	43.7	41.5	38.7	37.0	35.0	37.3	39.2	39.2	40.5
		80	63.0	64.5	65.8	66.8	63.7	61.3	59.0	63.3	59.7	57.0	53.7
		83	66.8	66.8	67.5	64.5	62.3	57.2	57.3	59.2	44.5	50.2	51.2
		95	52.0	54.2	51.0	49.7	45.5	44.3	39.5	46.5	44.2	45.8	42.0
		97	55.5	55.8	54.8	52.3	50.8	45.5	43.5	48.0	45.8	48.8	48.5
Ave............................			55.3	56.9	56.6	55.0	52.1	49.0	46.9	50.9	46.7	48.7	47.2
III	Limed grass & manganese	78	63.5	62.5	63.0	63.2	60.8	58.7	55.8	59.5	57.5	57.5	54.8
		84	49.5	54.2	51.0	50.3	48.3	42.7	42.3	46.7	42.0	45.0	40.0
		87	54.0	54.5	52.0	53.5	52.5	51.8	47.8	51.0	39.8	45.3	48.0
		88	68.2	71.0	65.8	66.7	62.8	61.2	57.3	60.5	60.2	60.0	59.5
		93	46.5	48.2	44.5	45.3	43.3	39.2	37.5	41.5	39.7	43.2	39.5
Ave............................			56.3	58.1	55.3	56.0	53.5	50.7	48.1	51.8	47.8	50.2	48.4

* D = died.

TABLE 2

Average weights of the goats, before and after the treatment

TREATMENT	PRE-GESTATION PERIOD		GESTATION PERIOD
	Before	2 months after	6 months after
	lb.	*lb.*	*lb.*
Unlimed grass.........................	55.4	55.0	47.0
Limed grass..........................	55.3	56.6	46.7
Limed grass plus manganese...........	56.3	55.3	47.8

in the blood, and the calcium and phosphorus in the serum of the goats in the three treatments.

The goats were healthy and vigorous at the end of the pre-gestation pe-

TABLE 3

Grass and minerals eaten by goats during pre-gestation and gestation periods (dry basis), pounds

TREATMENT	GOAT NO.	THIRD CROP	FOURTH CROP	TOTAL	CALCIUM (Ca)			PHOSPHORUS (P)			MANGANESE (Mn)			IRON (Fe)			MAGNESIUM (Mg)		
					a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Unlimed grass	81	162	116	278	.33	.34	.67	.45	.28	.73	.03	.04	.07	.03	.01	.04	.30	.24	.54
	90	176	163	339	.35	.47	.82	.48	.39	.87	.03	.04	.07	.04	.02	.06	.32	.33	.65
	91	156	152	308	.31	.44	.75	.43	.36	.79	.02	.04	.06	.03	.02	.05	.29	.31	.60
	92	130	124	254	.26	.36	.62	.36	.30	.66	.02	.03	.05	.03	.02	.05	.24	.26	.50
	94	162	158	320	.33	.46	.79	.45	.38	.83	.03	.04	.07	.03	.02	.05	.30	.33	.63
Total		786	713	1499	1.58	2.07	3.65	2.17	1.71	3.88	.13	.19	.32	.16	.09	.25	1.45	1.47	2.92
Limed grass	72	160	148	308	.54	.50	1.04	.49	.43	.92	.01	.03	.04	.03	.04	.07	.35	.31	.66
	80	195	170	365	.65	.57	1.22	.59	.50	1.09	.02	.03	.05	.03	.04	.07	.43	.35	.78
	83	194	154	348	.65	.52	1.17	.59	.45	1.04	.02	.03	.05	.03	.04	.07	.43	.32	.75
	95	183	132	315	.61	.45	1.06	.56	.39	.95	.02	.02	.04	.03	.03	.06	.41	.28	.69
	97	184	147	331	.62	.50	1.12	.56	.43	.99	.02	.03	.05	.03	.04	.07	.41	.31	.72
Total		916	751	1667	3.07	2.54	5.61	2.79	2.20	4.99	.09	.14	.23	.15	.19	.34	2.03	1.57	3.60
Limed grass plus manganese	78	188	196	384	.63	.66	1.29	.57	.57	1.14	.02	.04	.06	.03	.05	.08	.42	.41	.83
	84	159	139	298	.53	.47	1.00	.49	.41	.90	.01	.03	.04	.03	.03	.06	.35	.29	.64
	87	188	169	357	.63	.57	1.20	.57	.49	1.06	.02	.03	.05	.03	.04	.07	.42	.35	.77
	88	206	189	395	.69	.64	1.33	.63	.55	1.18	.02	.03	.05	.03	.05	.08	.46	.39	.85
	93	154	133	287	.52	.45	.97	.47	.39	.86	.01	.02	.03	.03	.03	.06	.34	.28	.62
Total		895	826	1721	3.00	2.79	5.79	2.73	2.41	5.14	.08	.15	.23	.15	.20	.35	1.99	1.72	3.71

a = third crop, b = fourth crop, c = total.

riod; but were skinny, bony, and weak at the end of the gestation period (see photo).   All of them, irrespective of treatment, showed at the end of gestation a reduction in red- and white-blood cells, in hematocrits, in hemoglobin, and in blood iron.   However, no change was evidenced in the calcium and phosphorus of the blood serum.

Of the total of fifteen goats; ten aborted, and one died.   Each of two goats, fed with limed grass, delivered a weak kid but did not produce milk

TABLE 4

Total dry grass and minerals eaten by the five goats, in each respective treatment, covering the eighth-month pre-gestation and gestation periods

TREATMENT	DRY GRASS EATEN			MINERALS EATEN				
	Pre-gestation period, 2-months	Gestation period, 6-months	Total, 8-months	Ca	P	Mn	Fe	Mg
	lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
Unlimed grass.........	344	1155	1499	3.65	3.88	0.32	0.25	2.92
Limed grass...........	419	1258	1677	5.61	4.99	0.23	0.34	3.60
Limed grass plus 203 milligrams of manganese supplied per os to 5 goats........	404	1317	1721	5.79	5.14	0.23	0.35	3.71

TABLE 5

Average intake of dry grass in pounds and minerals in grams per day per group of five goats of about 52 pounds each

TREATMENT	DRY GRASS EATEN	MINERALS EATEN				
		Ca	P	Mn	Fe	Mg
	lb.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Unlimed grass.......	6.17	6.8	7.2	0.60	0.47	5.5
Limed grass.........	6.90	10.5	9.3	0.43	0.64	6.7
Limed grass plus manganese........	7.08	10.8	9.6	0.43	0.65	6.9

even for the newborn.   Stylbestrol, at the rate of 5 milligrams per os, which was given to the goats to induce even ovulation, might have been the cause of upsetting their endocrine balance and affecting in general the health of the animal.   A marked increase was noticed in the calcium and phosphorus contents of the blood serum one-month after the stylbestrol application (table 7).

Iron combined with hemoglobin and uncombined are reported in table 8. This table contains data for 147 determinations made for iron and hemo-

globin in goat's blood taken monthly for a period of nine months, from each of the fifteen goats in the experiment. The color of the iron was developed by Wong's (5) method, and both, the iron and hemoglobin were determined separately in the spectrophotometer, as already reported. The combined iron in table 8 was calculated by multiplying the hemoglobin by the factor 3.35, as proposed by Wong (5) on the basis that

TABLE 6

Hematological data, iron in blood, calcium and phosphorus in blood serum, for goats in each treatment

TREATMENT	PERIOD	RED BLOOD CELLS PER CU. MM.	WHITE BLOOD CELLS PER CU. MM.	HEMAT-OCRITS	HEMO-GLOBIN	Fe IN BLOOD	Ca IN SERUM	P IN SERUM
		$\times 10^3$		% vol.	gm. %*	mg. %†	mg. %	mg. %
Unlimed grass	Before pre-gestation	17,626	19,980	31.9	10.5	48.2	11.4	4.9
	End of pre-gestation	16,894	15,980	30.2	10.1	37.1	12.5	4.0
	End of gesta-tion	15,043	13,475	25.3	8.2	35.4	13.8	4.2
Limed grass	Before pre-gestation	19,680	19,560	34.2	11.5	51.2	10.6	5.5
	End of pre-gestation	17,646	18,880	32.5	10.7	39.0	12.2	4.2
	End of gesta-tion	14,662	13,790	23.7	7.9	34.2	14.1	5.2
Limed grass plus man-ganese	Before pre-gestation	20,508	16,490	35.7	11.8	51.9	9.9	6.2
	End of pre-gestation	18,206	15,690	33.0	11.1	40.1	10.2	5.9
	End of gesta-tion	12,624	13,910	22.5	7.6	34.1	13.9	5.2

* Grams hemoglobin per 100 ml. blood.
† Milligrams Fe per 100 ml. blood.

hemoglobin contains 0.0335 per cent iron as Fe. Results in table 8 reveal that considerable of the iron in the blood is not combined with hemoglobin. The uncombined iron varied from 0.1 to 19.9 milligrams per 100 milliliters of blood in 129 blood tests. In 18 tests the iron calculated from the hemoglobin was from 0.79 to 13.0 milligrams per 100 milliliters of blood higher than that found. The low iron content of the blood and the iron deficit in the hemoglobin occurred at regular intervals; the first, at the fifth month period of the experiment; the second, at the ninth month period.

Wong's proposal for calculating hemoglobin from the iron content of the blood does not give, therefore, a true value for hemoglobin, nor does the calculation of blood iron from the hemoglobin content gives a true value for the iron in blood.
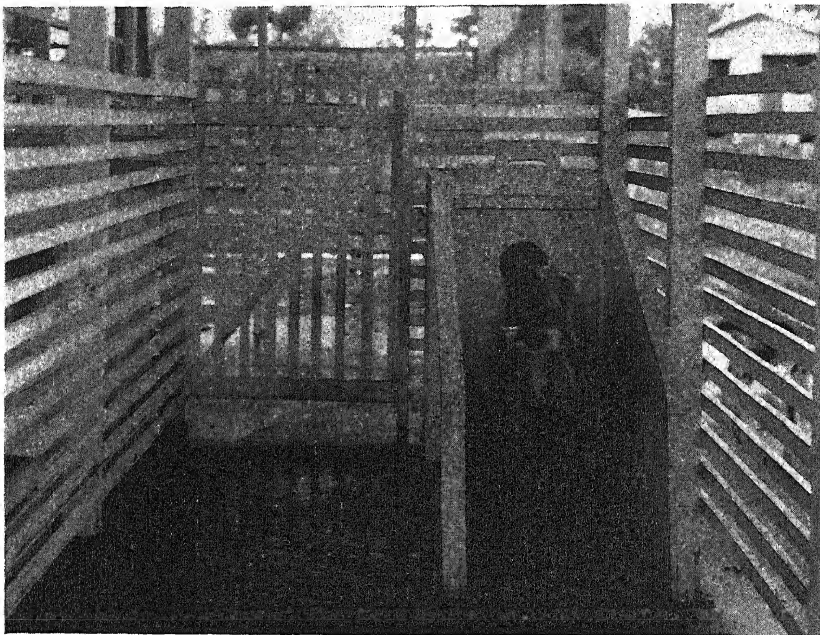
A GOAT IN ITS PEN EATING GRASS FROM THE FEEDING BOX
Note poor physical appearance of goat during the gestation period

TABLE 7

Mean calcium and phosphorus contents of blood serum from fifteen female goats before and after stylbestrol application

MINERAL	STYLBESTROL APPLICATION		
	Before	One month after	Two months after
	%	%	%
Calcium, Ca......................	11.6	17.8	11.6
Phosphorus, P....................	4.7	6.2	5.7

It has been mentioned that the animals sustained their weight and were healthy and vigorous at the end of the pre-gestation period; but were skinny, bony, and weak at the end of the gestation period. A reduction in red and white blood cells, in hematocrits, in hemoglobin and blood iron,

TABLE 8

Hemoglobin; iron calculated from hemoglobin, iron determined, and uncombined iron, in blood samples taken monthly for a period of nine months from fifteen female goats

TREATMENT	GOAT NO.	DATE	HEMOGLOBIN	CALCULATED Fe Hb $\times$ 3.35	DETER-MINED Fe.	UNCOM-BINED Fe
		mo.	*g/100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
1. Goats receiving unlimed grass	81	0	10.20	34.17	46.8	12.63
		1	9.96	33.37	42.7	9.33
		2	9.24	30.95	35.3	4.35
		3	9.00	30.15	35.6	5.45
		4	9.62	32.23	36.8	4.57
		5	9.80	32.83	34.3	1.47
		6	8.32	27.87	33.2	5.33
		7	6.00	20.10	23.3	3.20
		8	—	—	—	—
		9	—	—	—	—
	90	0	11.50	38.53	49.2	10.67
		1	10.20	34.17	44.8	10.63
		2	9.82	32.90	36.2	3.30
		3	9.96	33.37	38.5	5.13
		4	8.30	27.81	35.2	7.39
		5	9.20	30.82	31.4	.58
		6	8.64	28.94	33.2	4.26
		7	6.60	22.11	25.7	3.59
		8	7.44	24.92	33.6	8.68
		9	7.40	24.79	17.5	−7.29
	91	0	9.20	30.82	50.7	19.88
		1	8.66	29.01	43.7	14.69
		2	8.66	29.01	32.5	3.49
		3	10.08	33.77	39.5	5.73
		4	8.90	29.82	38.2	8.38
		5	9.00	30.15	31.2	1.05
		6	8.20	27.47	32.4	4.93
		7	7.38	24.72	29.5	4.78
		8	8.38	28.07	37.5	9.43
		9	8.00	26.80	17.7	−9.10
	92	0	10.50	35.18	47.0	11.82
		1	10.40	34.84	44.8	9.96
		2	11.00	36.85	40.5	3.65
		3	9.98	33.43	38.5	5.07
		4	9.02	30.22	35.3	5.08
		5	9.70	32.50	33.0	.50
		6	8.00	26.80	32.5	5.70
		7	7.78	26.06	28.0	1.94
		8	7.90	26.47	33.8	7.33
		9	7.94	26.60	16.8	−9.80

TABLE 8—*Continued*

TREATMENT	GOAT NO.	DATE	HEMOGLOBIN	CALCULATED Fe Hb × 3.35	DETER-MINED Fe	UNCOM-BINED Fe
		mo.	*g/100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
1. Goats receiving unlimed grass —*Continued*	94	0	11.00	36.85	47.5	10.65
		1	11.54	38.66	48.5	9.84
		2	11.54	38.66	40.8	2.14
		3	11.22	37.59	43.3	5.71
		4	9.68	32.43	38.7	6.27
		5	10.80	36.18	32.6	−3.58
		6	10.02	33.57	41.6	8.03
		7	8.16	27.34	—	—
		8	9.20	30.82	36.5	5.68
		9	9.00	30.15	22.8	−7.35
2. Goats receiving limed grass	72	0	12.10	40.54	55.5	14.96
		1	10.80	36.18	43.0	6.82
		2	10.40	34.84	37.7	2.86
		3	11.00	36.85	41.0	4.15
		4	10.16	34.04	42.0	7.96
		5	9.60	32.16	33.6	1.44
		6	9.02	30.22	35.5	5.28
		7	7.00	23.45	28.7	5.25
		8	8.30	27.81	36.4	8.59
		9	7.76	26.00	13.0	−13.00
	80	0	11.80	39.53	49.5	9.97
		1	10.56	35.38	42.5	7.12
		2	11.30	37.86	41.3	3.44
		3	11.38	38.12	42.5	4.38
		4	9.96	33.37	39.7	6.33
		5	10.00	33.50	33.6	.10
		6	9.84	32.96	36.8	3.84
		7	7.46	24.99	40.0	15.01
		8	9.00	30.15	42.3	12.15
		9	8.24	27.60	19.2	−8.40
	83	0	11.00	36.85	54.3	17.45
		1	10.80	36.18	42.7	6.52
		2	10.20	34.17	36.7	2.53
		3	10.80	36.18	41.0	4.82
		4	10.20	34.17	43.4	9.23
		5	10.80	36.18	36.4	.22
		6	9.24	30.95	35.7	4.75
		7	6.80	22.78	24.2	1.42
		8	7.20	24.12	28.0	3.88
		9	7.00	23.45	17.3	−6.15

TABLE 8—*Continued*

TREATMENT	GOAT NO.	DATE	HEMOGLOBIN	CALCULATED Fe Hb × 3.35	DETER-MINED Fe	UNCOM-BINED Fe
		mo.	*g/100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
2. Goats receiving limed grass—*Continued*	95	0	11.00	36.85	49.1	12.25
		1	11.10	37.19	47.0	9.81
		2	10.60	35.51	40.0	4.49
		3	9.92	33.23	39.5	6.27
		4	7.86	26.33	32.7	6.37
		5	9.00	30.15	30.3	.15
		6	7.50	25.13	31.0	5.87
		7	6.80	22.78	28.1	5.32
		8	7.04	23.58	30.5	6.92
		9	6.16	20.64	15.2	−5.44
	97	0	11.40	38.19	47.5	9.31
		1	12.56	42.08	54.0	11.92
		2	10.76	36.05	39.4	3.35
		3	10.08	33.77	39.8	6.03
		4	9.62	32.23	38.7	6.47
		5	9.60	32.16	31.5	−.66
		6	9.50	31.83	34.4	2.57
		7	7.38	24.72	27.8	3.08
		8	—	—	33.8	—
		9	7.90	26.47	18.7	−7.77
3. Goats receiving limed grass plus manganese	78	0	13.20	44.22	61.5	17.28
		1	12.20	40.87	50.5	9.63
		2	12.40	41.54	45.0	3.46
		3	12.18	40.80	46.2	5.40
		4	9.80	32.83	44.0	11.17
		5	12.10	40.54	39.8	−.74
		6	11.06	37.05	41.5	4.45
		7	8.56	28.68	33.8	5.12
		8	8.60	28.81	30.0	1.19
		9	7.92	26.53	18.8	−7.73
	84	0	10.40	34.84	47.6	12.76
		1	12.70	42.55	54.8	12.25
		2	12.20	40.87	43.5	2.63
		3	12.38	41.47	44.8	3.33
		4	8.96	30.02	35.8	5.78
		5	10.40	34.84	35.6	.76
		6	10.20	34.17	40.4	6.23
		7	8.56	28.68	32.8	4.12
		8	7.60	25.46	43.3	17.84
		9	3.60	12.06	5.5	−6.56

TABLE 8—*Continued*

TREATMENT	GOAT NO.	DATE	HEMOGLOBIN	CALCULATED Fe Hb × 3.35	DETER- MINED Fe	UNCOM- BINED Fe
		mo.	*g/100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
3. Goats receiving limed grass plus manganese— *Continued*	87	0	11.04	36.98	49.0	12.02
		1	10.10	33.84	40.0	6.16
		2	9.36	31.36	36.2	4.84
		3	8.40	28.14	34.6	6.46
		4	9.00	30.15	37.8	7.65
		5	9.40	31.49	30.7	− .79
		6	8.10	27.14	31.7	4.56
		7	6.18	20.70	24.4	3.70
		8	5.86	19.63	25.3	5.67
		9	5.14	17.22	12.5	−4.72
	88	0	12.80	42.88	52.5	9.62
		1	11.80	39.53	47.0	7.47
		2	11.40	38.19	38.5	.31
		3	9.92	33.23	39.6	6.37
		4	10.06	33.70	38.7	5.00
		5	9.40	31.49	34.5	3.01
		6	8.48	28.41	31.4	2.99
		7	7.46	24.99	30.3	5.31
		8	8.60	28.81	37.2	8.39
		9	7.60	25.46	18.6	−6.86
	93	0	11.50	38.53	49.0	10.47
		1	10.90	36.52	43.5	6.98
		2	10.34	34.64	37.4	2.76
		3	9.60	32.16	89.0	6.84
		4	9.80	32.83	38.5	5.67
		5	9.90	33.17	33.6	.43
		6	8.90	29.82	35.1	5.28
		7	7.60	25.46	29.3	. 3.84
		8	7.20	24.12	34.5	10.38
		9	6.40	21.44	15.2	−6.24

was also reported for the gestation period.   It has also been mentioned that two thirds of the goats aborted and that the two goats delivering weak kids produced no milk.   Presumably the stylbestrol application upset the endocrine balance and affected the general health of the animals.   All indications pointed to malnutrition of the goats during gestation, probably due to an inadequate protein intake.

A composite sample of the grass fed to the animals was not collected during the pre-gestation and gestation periods.   The mineral intake for the goats was calculated from the mineral composition of the grass at time of sampling (table 4).   The protein intake (table 9) was calculated similarly.

During the pre-gestation period the goats were fed exclusively with grass from the third crop (1) that received a nitrogen application at the rate of 500 pounds of ammonium sulphate per acre. The protein content of this grass at time of sampling, or 36 days after nitrogen application, was 11.2 per cent.

During the gestation period the goats were fed 72 days with high protein grass from the third crop and 110 days with a low protein grass from the fourth crop that received no nitrogen fertilization.

The daily intake of protein per goat for the pre-gestation period was estimated to be 0.143 pounds or about 65 grams, and for the gestation period was .089 pounds or about 40 grams (table 9). The goats ate per day about the same amount of grass in the pre-gestation and gestation periods, but the

TABLE 9

Estimated protein intake per goat per day during the pre-gestation and gestation periods

| PERIOD | TIME OF PERIOD | AMOUNT OF GRASS EATEN PER GOAT PER | | PROTEIN CONTENT OF GRASS AT TIME OF SAMPLING | ESTIMATED AMOUNT OF PROTEIN EATEN PER GOAT PER DAY |
		Period	Day		
	days	*lbs.*	*lbs.*	%	*lb.*
Pre-gestation	61	77.8	1.28	11.2	0.143
Gestation	72	98.4	1.37	11.2	0.153
	110	150.3	1.37	3.5	0.048
	182	248.7	1.37		0.089

daily intake of protein was 25 grams less in the gestation period. This protein deficiency might have been the cause for the malnutrition of the goats during the gestation period.

CONCLUSION

Female goats kept in their pens, and fed exclusively with a mixture of un-limed and limed Para or "Malojillo" grass (*Panicum purpurascens*) and Carib or "Malojilla" grass (*Eriochloa polystachya*), suffered malnutrition effects during the six-month gestation period. The low normal protein content of the grass mixture, around 3.5 per cent, was probable the main factor involved. Malnutrition was unnoticed during the two-month pre-gestation period because, due to adequate and timely nitrogen fertilization the protein content of the grass mixture was raised to a higher level, around 11 per cent.

Nutrition studies involving mineral in animal blood require that the animal be fed with an adequate protein level during the whole experimental period.

SUMMARY

This paper reports spectrophotometric methods and transmittance-concentration curves for hemoglobin and iron in blood and phosphorus in blood serum and a photocolorimeter method and curve for calcium in blood serum.

Fifteen cross-bred virgin female goats were randomized in groups of five and fed according to three treatments: unlimed grass, limed grass, and limed grass plus manganese per os.   Amounts of grass fed to each animal and their monthly weights were recorded for a one-month prefeeding period, a two month pre-gestation period, and a six-month gestation period.   Blood samples were also drawn monthly from each animal for determination of hemoglobin, iron, hematocrits, red blood cells and white blood cells in blood, and calcium and phosphorus in blood serum.

The data are reported in nine tables.   The important results are as follows:

1. There was no significant increase in the weight of the goats for the two-month pre-gestation period; however, there was a highly significant decrease in weight after the six-month gestation period.
2. There were significant differences for the pre-gestation period between the grass intake of the goats under the three treatments; the goats fed with unlimed grass ate less than those fed with limed grass. However, for the gestation period, the differences for the grass intake between the treatments was not significant.
3. The average intake of dry grass in pounds, and mineral in grams per day per group of five goats, covering the 243 days of the pre-gestation and gestation periods, are reported in table 5.
4. There were no significant differences in the pre-gestation between the mean red or white blood cells, the hematocrits, the hemoglobin and iron in the blood, and the calcium and phosphorus in the serum of the goats in the three treatments.
5. The goats were healthy and vigorous at the end of the pre-gestation period; but were skinny, bony and weak at the end of the gestation period (see photo).
6. The goats at the end of gestation, showed a reduction in red and white blood cells, in hematocrits, in hemoglobin, and in blood iron. However, no change was evidenced in the calcium and phosphorus of the blood serum.
7. Of the total of fifteen goats; ten aborted, and one died.   Each of two goats delivered a weak kid, but produced no milk.
8. Stylbestrol, applied at the rate of 5 milligrams per os to each goat, to induce even ovulation, might have been the cause of upsetting the endocrine balance and affecting the health of the animal.   A

marked increase was noticed in the calcium and phosphorus contents (table 7) of the blood serum after the stylbestrol applications.

9. Not all of the iron present in the blood is combined with hemoglobin (table 8).

10. The estimated protein intake per goat during the pre-gestation period was calculated to be about 65 grams per day (table 9), while that during the gestation period was about 40 grams per day. This protein deficit in the gestation period might have been an important factor causing malnutrition in the goats.

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LABORATORY RECOMMENDATION OF LIME TO AN ACID SOIL CHECKS WITH EXPECTED pH CHANGES

ALFONSO RIERA

INTRODUCTION

The agricultural value of about one million acres of acid soils in the humid area of Puerto Rico may be improved with the application of lime. The textures of these soils vary from the sands to the clays. Their organic matter has been reported (1) to vary from 1.0 per cent in the sandy soils to 51.5 per cent in the mucks. The buffer capacity of these soils is variable. A laboratory method for determining the lime requirement, which will be applicable to field conditions, is, therefore, of paramount importance.

Dr. B. G. Capó, Head of the Department of Agronomy and Horticulture, worked as a Soil Chemist in the Department of Soils from 1936 to 1942. While he was working in pot studies with soil-sand mixtures, using Hegari sorghum as a plant index to determine the available major nutrients in the soils, he adopted the following lime-requirement method to bring the acid soils to a convenient pH value.

LABORATORY METHOD

The air-dried soil samples are ground to pass a 1 mm. sieve. Calcium carbonate c.p. Baker Analized, is used as the lime source. Five portions of 20 grams of soil are weighed and placed in each of five 250 ml. beakers. The amounts of calcium carbonate added respectively, to each beaker, are 20, 40, 60, 80 and 100 milligrams corresponding to 1, 2, 3, 4 and 5 tons of calcium carbonate per acre. One hundred and fifty cubic centimeters of distilled water are added to each beaker. The mixture is stirred for 3 hours in a Ross-Kershaw apparatus, described as ⌗9235 of Arthur H. Thomas catalog. The pH values of the supernatant liquids are determined and plotted on coordinate paper against the tons of calcium carbonate. The amount of limestone necessary to bring the pH of the supernatant liquid to the desired pH value is estimated from the corresponding curve.

FIELD RESULTS

"Fajardo clay" is an acid soil type derived from ashy shale. This soil type is well distributed in the terraces of the Experiment Station farm. Soil samples, at six-inch depths were taken on June 1943 from each of nine plots of "Fajardo clay" for the lime requirement tests. The amount of limestone necessary to bring the soil of each plot up to pH 6.5 was cal-

culated from the corresponding pH-lime requirement curve as described above.

The required amount of commercial ground limestone was applied to each of these plots and "malojillo" (Para) grass was planted on them on July 1943. On October 1944, soil samples were taken again at each of these plots, for pH determinations with the results that appear on table 1.

TABLE 1

Values of pH before and after liming

PLOT NUMBER	pH VALUE BEFORE LIMING	CALCIUM CARBONATE TO RAISE UP TO pH 6.5	pH VALUE 15 MONTHS AFTER LIMING
		tons/acre	
1	4.4	10.0	6.2
4	4.4	10.0	6.3
5	4.1	8.0	6.4
8	4.6	10.0	6.6
9	4.7	10.0	6.7
12	4.9	10.0	6.6
13	5.5	8.0	6.9
18	4.2	12.0	5.2
20	4.6	10.0	6.1

The difference between the mean pH values does not differ statistically from the desired value 6.5.

SUMMARY

A laboratory method for lime requirement in soils is presented. Lime was applied to the acid soil of nine plots of a field experiment at the rate found by this method. The pH changes fifteen months later did not differ statistically from the ones expected.

RESUMEN

Se expone aqui un método de laboratorio relacionado con la cal que necesitan los terrenos. Se aplicó cal a los terrenos ácidos de nueve parcelas que constituyeron el campo de un experimento, en la proporción determinada por dicho método. Los cambios pH, quince meses después, no se diferenciaron estadisticamente, de aquellos que se habían esperado.

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LACK OF RESPONSE OF SUGARCANE TO APPLICATIONS OF PHOSPHORUS IN PUERTO RICO

J. A. BONNET, B. G. CAPÓ AND A. RIERA

INTRODUCTION

In the last decade, 1934–1944, sugarcane, the most important economic crop of Puerto Rico, has occupied an average of 303,678 acres. The peak of total production was reached with the 1941–1942 crop which amounted to 10,010,132 tons of cane and yielded 1,147,589 tons of sugar. The Association of Sugar Producers of Puerto Rico reports that in 1941, from January to December, 141,000 tons of fertilizer raw materials were imported—80,000 tons ammonium sulphate, 36,000 tons superphosphate and 25,000 tons potash salts—from which about 80 per cent or 112,800 tons were used for sugarcane. The normal application for fall and spring plantings of sugarcane in Puerto Rico is six bags of 200 pounds each, per acre; and for ratoons, 4 bags per acre. Except for certain sections of the arid region of the South coast of the Island where ammonium sulphate is applied only, a complete fertilizer is used as a general practice. From July 1941 to June 1942, both inclusive, 92,975 tons of complete fertilizers (5) were applied to sugarcane in Puerto Rico. The available phosphoric acid in those fertilizers varied between four and ten per cent and added up to 5,658 tons P_2O_5. At the estimated price of $28.00 per ton of superphosphate containing 20 per cent available phosphoric acid, the consumption of phosphate fertilizer for the 1941–42 crop amounted to $792,120. The investment in phosphorus for sugarcane for the last 24 years (1920–1944) is estimated to be around ten million dollars.

The official fertilizer formulae for sugarcane approved by the War Production Board for manufacture in 1944–45, on the basis of NH_3, P_2O_5 and K_2O, are: 10-6-9, 12-4-9, 14-6-5, 14-6-8, 14-3-8, 14-0-7, 14-0-11. The demand for the formulae containing no phosphorus, however, has been practically negligible.

It is of economic importance, therefore, to determine if the continuous addition of phosphorus to the fertilizer is necessary for the sugarcane crop. It is also of importance to know if the placement of the fertilizer has any effect upon the response of the cane plant to applications of phosphorus.

EXPERIMENTAL WORK

A number of field experiments has been conducted in the past few years in which the need of phosphorus applications to maintain or increase

sugar yields has been under study. In these experiments, phosphoric acid fertilizers have been tried in different amounts, the rate of the phosphoric acid applications varying from zero to a maximum of four hundred P_2O_5 per acre. The actual rates of application as well as the mean cane yields obtained in these experiments are presented in tables 1 and 2.

The experiments were performed with several sugarcane varieties and they were established in the following 14 soil types (4) representing 84,224 acres of the most important sugarcane producing soils: "Toa silty clay loam", "Toa silty clay", "Toa clay", "Coloso silt loam", "Coloso silty clay loam", "Coloso silty clay", "Aguirre clay", "Vayas clay", "Vega Baja silty clay", "Mercedita clay", "Mabí clay", "Moca loam", "Vega Alta clay loam" and "Cataño sandy loam". The description of the soil series to which these soil types belong is as follows:

Toa. Series of the well-drained soils of the river flood plains in the humid area, derived from materials washed from the limestone and tuffaceous hills. They are neutral or slightly acid in reaction and are high in bases and plant nutrients. They are friable, brown soils from the surface to a depth of below four feet. The productivity rating of the loam to clay types is 1, i.e., they are rated among the best soils.

Coloso. Series of the poorly drained soils of the river flood plains in the humid area, derived from neutral, fine textured materials of volcanic rocks and limestone. They are poorly drained associates of the "Toa" series. They are deep, stone free, highly fertile, neutral, and plastic. Most areas have a heavy texture, a high water table, a dark surface soil, and a mottled-gray, bluish-gray, and rust-brown subsoil. The productivity of the heavy types is rated between 1 and 2.

Aguirre. Series of the poorly drained soils of the river flood plains in the arid area, that occupy areas that are transitional in character between the soils of the well drained river flood plains or alluvial fans, and the poorly drained soils of the coastal lowlands. In a cultivated field, "Aguirre clay" has a 10 or 12 inch very dark, grayish-brown and gray plastic sticky clay that continues to a depth ranging from 30 to 36 inches. The upper part of the substratum is a mottled-gray, rust-brown, and yellowish-brown, medium plastic, wet silty clay, that in places contains some medium-sized gravel. At a depth ranging from 5 to 6 feet is the substratum of bluish-gray, plastic, sticky wet clay. This layer continues to considerable depths and has characteristics of estuarine deposits. Nearly all of the layers in the profile contain free lime; many areas contain salts, chiefly sodium carbonate, that limit crop production. Areas that contain less than 0.2 per cent of salts within the first four feet are used under irrigation for the production of sugarcane. It has a productivity rating of 2. The

TABLE 1

Percentage increases in cane yields obtained when phosphoric acid applications were made

CROP NO.	SOIL TYPE AND LOCATION	CANE VARIETY	KIND OF CROP AND YEAR	FERTILIZERS APPLIED PER ACRE			CANE YIELDS PER ACRE	IN-CREASE IN YIELD
				NH₃	P₂O₅	K₂O		
				cwts.	cwts.	cwts.	tons	per cent
1	Coloso silty clay, Toa Baja	BH-10(12)	Plant Cane 1937–1939	4 4	0 4	4 4	69.3 70.2	1.3
2	" "	BH-10(12)	First Ratoon 1939–40	4 4	0 4	4 4	50.7 46.7	−7.9
3	" "	BH-10(12)	Second Ratoon 1940–41	4 4	0 4	4 4	37.6 40.8	8.5
			Average of 3 crops	4 4	0 4	4 4	52.5 52.6	0.0
4	Coloso silt loam Toa Baja	BH-10(12)	Plant Cane 1938–40	4 4	0 4	4 4	68.4 71.2	4.1
5	" "	BH-10(12)	First Ratoon 1940–41	4 4	0 4	4 4	49.8 46.9	−5.8
			Average of 2 crops	4 4	0 4	4 4	59.1 59.1	0.0
6	" "	POJ-2878	Plant Cane 1938–40	4 4	0 4	4 4	65.3 65.0	−0.5
7	" "	POJ-2878	First Ratoon 1940–41	4 4	0 4	4 4	62.4 60.3	−3.4
			Average of 2 crops	4 4	0 4	4 4	63.9 62.7	−1.9
8	" "	M-28	Plant Cane 1938–40	4 4	0 4	4 4	66.7 67.1	0.6
9	" "	M-28	First Ratoon 1940–41	4 4	0 4	4 4	45.5 41.6	−8.6
			Average of 2 crops	4 4	0 4	4 4	56.1 54.4	−3.0
10	" "	M-275	Plant Cane 1938–40	4 4	0 4	4 4	67.2 68.1	1.3

TABLE 1—*Continued*

CROP NO.	SOIL TYPE AND LOCATION	CANE VARIETY	KIND OF CROP AND YEAR	FERTILIZERS APPLIED PER ACRE			CANE YIELDS PER ACRE	IN-CREASE IN YIELD
				NH₂	P₂O₅	K₂O		
				cwts.	cwts.	cwts.	tons	per cent
11	Coloso silt loam Toa Baja	M-275	First Ratoon 1940–41	4 4	0 4	4 4	58.4 60.9	4.3
			Average of 2 crops	4 4	0 4	4 4	62.8 64.5	2.7
12	Aguirre clay, Salinas	BH-10(12)	Plant Cane 1940–42	4.5 4.5	0 3	4 4	45.1 41.5	−8.0
13	" "	POJ-2878	Plant Cane 1940–42	4.5 4.5	0 3	4 4	42.8 45.0	5.1
14	Mabi clay, Juncos	POJ-2878	Plant Cane 1940–42	4.5 4.5	0 3	4 4	75.4 79.0	4.8
15	Mercedita clay, Ponce	BH-10(12)	Plant Cane 1940–42	4.5 4.5	0 3	4 4	97.0 95.2	−1.9
16	" "	POJ-2878	Plant Cane 1940–42	4.5 4.5	0 3	4 4	108.8 102.1	−6.2
17	Toa silty clay, Manatí	M-275	Plant Cane 1941–43	2.325 2.325	0 1.55	2.325 2.325	94.9 105.0	10.6
18	Toa silty clay loam, Manatí	BH-10(12)	Plant Cane 1942–43	3 3	0 2	3 3	74.2 73.7	−0.7
19	Coloso silt loam, Fajardo	BH-10(12)	Plant Cane 1937–39	4 4	0 4	4 4	89.8 82.0	−8.7
20	" "	BH-10(12)	First Ratoon 1939–40	4 4	0 4	4 4	48.4 53.9	11.4
			Average of 2 crops	4 4	0 4	4 4	69.1 68.0	−1.6
21	Moca loam, Toa Baja	POJ-2878	Plant Cane 1939–40	3 3	0 3	3 3	40.4 43.5	7.7
22	Coloso silty clay loam, Naguabo	BH-10(12)	First Ratoon 1937–39	3 3	0 3	3 3	81.9 79.9	−2.4
23	Cataño sandy loam, Añasco	POJ-2878	Plant Cane 1937–38	0.9 0.0	0 0.9	0.9 0.9	47.4 49.9	5.3
24	Toa clay, Hormigueros	POJ-2878	Plant Cane 1937–38	0.9 0.9	0 0.9	0.9 0.9	36.9 37.4	1.4

land is difficult to plow and cultivate because when wet it is plastic and sticky, and when dry it is hard and cloddy.

Vayas. Series that occupy the more poorly drained level areas along

TABLE 2

Percentage increases in cane yields obtained with increases in phosphoric acid applications above the minimum 0.20 cwt. P_2O_5 per acre applications

TREATMENTS			POJ-2878, VEGA ALTA CLAY LOAM, RÍO PIEDRAS, FIRST RATOON, 12 MONTHS. 1943–44		BH-10(12), VAYAS CLAY, SANTA RITA			
					First ratoon, 12 months. 1942-43		New planting of plant cane, 10 months. 1943-44	
NH_3	P_2O_5	K_2O	Yield	Per cent increase	Yield	Increase	Yield	Increase
cwt./A.	cwt./A.	cwt./A.	tons/A.		tons/A.	%	tons/A.	%
1.25	0.20	0.90	39.9		33.8		24.4	
1.25	0.80	0.90	38.8	−2.8	30.0	−11.2	24.1	−1.2
1.25	0.20	1.80	37.0		30.5		22.5	
1.25	0.80	1.80	39.1	5.7	29.9	−2.0	23.3	3.6
1.25	0.20	2.70	39.6		27.0		25.5	
1.25	0.80	2.70	40.6	2.5	31.4	16.3	24.0	−5.9
2.00	0.20	0.90	36.9		29.9		21.0	
2.00	0.80	0.90	37.0	0.3	32.5	8.7	25.3	20.5
2.00	0.20	1.80	38.8		29.3		21.0	
2.00	0.80	1.80	39.3	1.3	32.8	11.9	26.9	28.1
2.00	0.20	2.70	38.0		30.1		23.1	
2.00	0.80	2.70	37.9	−0.3	29.1	−3.3	26.9	16.5
2.75	0.20	0.90	37.9		32.3		24.4	
2.75	0.80	0.90	38.6	1.8	30.6	−5.3	22.1	−9.4
2.75	0.20	1.80	41.3		34.3		26.6	
2.75	0.80	1.80	37.1	−10.2	27.5	−19.8	20.5	−22.9
2.75	0.20	2.70	36.1		32.8		26.8	
2.75	0.80	2.70	37.9	5.0	29.9	−8.8	23.6	−11.9
3.50	0.20	0.90	37.9		29.9		23.5	
3.50	0.80	0.90	34.6	−8.7	33.3	11.4	28.1	19.6
3.50	0.20	1.80	40.0		29.5		26.3	
3.50	0.80	1.80	35.5	−11.3	31.4	6.4	24.4	−7.2
3.50	0.20	2.70	37.6		32.9		26.5	
3.50	0.80	2.70	38.5	2.4	30.3	−7.9	27.5	3.8
Average with 0.20 cwt. P_2O_5 p/A.			38.4		31.0		24.3	
Average with 0.80 cwt. P_2O_5 p/A.			37.9	−1.3	30.7	−1.0	24.7	1.6

the river flood plains in the semiarid south coast section. "Vayas clay" has a brown or dark-brown clay in the surface and a mottled-gray or rust-brown and yellowish-brown layer beginning at a depth of about 30 inches and continuing to considerable depths. The average upper limit of the

water table is about 30 inches. Nonalkali areas have a productivity rating
of 1. When wet it is plastic, and when it dries large cracks appear on the
surface.

Vega Baja. Series of the poorly drained soils of the river flood plains
in the humid region. They occupy bordering or intergrade areas between
the alluvial soils and the coastal plain soils. They are slightly above
normal overflow, but during exceptionally high water are flooded. The
surface soil of the silty-clay type, to a depth of eight or ten inches, is acid,
friable and granular. It has a light-brown or grayish-brown color. This
layer changes abruptly to a plastic, medium compact, mottled, yellowish-
brown, gray and red silty clay or clay subsoil, which continues to consider-
able depth, and becomes more definitely mottled and more acid with
depth. Its productivity is rated between 2 and 3.

Mercedita. Series of the inner plain soils in the arid region. The 12
inch surface soils of Mercedita clay consist of brown or dark-grayish-
brown, granular calcareous clay, which is very plastic and waxy when wet.
The subsoil is yellowish-brown or light olive-brown, medium compact,
plastic calcareous clay, ranging from 10 to 15 inches in thickness. Below
this layer is a very limy, friable, light-yellow silty material and soft lime-
stone, which continues below a depth of 5 feet. Some areas contain
harmful quantities of salts. Many areas are affected with lime chlorosis.
Its productivity is rated at 2.

Mabí. Series of the inner plain soils in the humid region. "Mabí
clay" occurs on long low gentle slopes, in close association with the "Mú-
cara" and related brown, shallow soils of the uplands. It is derived from
tuffaceous material, partly residual, and in part colluvial and alluvial.
It has a grayish-brown, neutral, plastic heavy, clay surface soil, about
eight or ten inches thick, which has good tilth when properly plowed and
cultivated. The subsoil is a yellowish-brown, plastic sticky, neutral,
heavy clay, streaked with rust-brown and gray material. This layer
gradually changes, at a depth ranging from 30 to 40 inches, to friable
brown and yellowish-brown silty clay loam that crumbles readily between
the fingers. The material grades into desintegrated tuffaceous rock ma-
terial at a depth ranging from four to 12 feet. Small fine white specks of
the rock material occur in all layers. Its productivity is rated at 3.

Moca. Series of the inner plain soils in the humid area. "Moca loam"
occurs on low sloping areas near the limestone hills. It has eight or ten-
inch, acid, brown or dark-brown friable loam, which abruptly changes to
heavy plastic, sticky, acid silty clay, or clay that is mottled red, gray,
and brown. This layer continues downward for many feet. All layers
contain a few rounded pieces of gravel or small rocks. Its productivity is
rated at 6.

Vega Alta. Series of the friable soils of the coastal plains in the humid

region. "Vega Alta clay loam" has a friable, brown or light-brownish-gray, acid surface, about eight inches thick, underlain by a reddish-brown, heavy, slightly plastic clay layer about 10 or 12 inches thick. This layer rests on more compact, mottled, brown, red, and gray clay, which continues to great depths before limestone is reached. Its productivity is rated at 5.

Cataño. Series belonging to the soils of the coastal lowlands in the humid region. It occurs as a narrow strip paralleling the sea, a short distance inland. It has a 10 or 12 inch surface soil of grayish-brown or dark-grayish-brown, loose noncoherent loamy sand, alkaline in reaction. This layer is underlain by a lighter colored and lighter textured calcareous subsoil about two feet thick. The substratum, to a depth ranging from 10 to 15 feet, is loose, friable sand. Its productivity is rated at 6.

The mean contents of phosphoric acid available in 1% citric acid, for various samples of soil types corresponding to the above soil series have been reported elsewhere (2.3) and are as follows:

SOIL SERIES	SAMPLES ANALYZED	AVAILABLE P_2O_5
Toa	21	.012
Coloso	18	.015
Aguirre	1	.041
Vayas	1	.072
Vega Baja	4	.013
Mercedita	1	.001
Mabi	11	.005
Moca	3	.003
Vega Alta	5	.008
Cataño	8	.017

The mean contents of total phosphoric acid of 7 samples of the Toa series and of 6 samples of the Coloso series were found (2) to be .113 and .154 per cent, respectively.

In general, the soil series of the arid region of Puerto Rico (3) contain about twice as much available phosphoric acid, soluble in 1% citric acid, than the soil series of the humid region. The mean difference is significant at the 1% point. The figures are as follows:

PER CENT AVAILABLE P_2O_5 IN SOIL SERIES OF		
Humid Area	Arid Area	Mean Difference
$.0079 \pm .0007$	$.0173 \pm .0030$	$.0094 \pm .002$

Highly significant.

<center>EXPERIMENTAL RESULTS</center>

Table 1 shows the yields of cane, in tons per acre, obtained in twenty-four crops of various sugarcane field experiments, both without and with a heavy application of phosphoric acid. Heavy applications of nitrogen and potash were added in all cases to insure the maximum possible responses from the phosphoric acid applications.

The first eighteen crops are from experiments performed in cooperation between the Soils and Agronomy Departments of the Agricultural Experiment Station of the University of Puerto Rico. Crops number 19 and 20 belong to an experiment carried out by Mr. Juan E. Veve, while at the Central Fajardo Experiment Station; crop no. 21 is from an experiment performed by Mr. Fernando Chardón at Central Constancia; crop no. 22 is from an experiment carried out by Mr. H. A. Nadler, Jr. of Eastern Sugar Associates; and crops nos. 23 and 24 were reported (1) by the Puerto Rico (Mayaguez) Agricultural Experiment Station.

Table 2 presents data relative to three crops of two long-time sugarcane field tests which are being performed by the Soils Department of the Agricultural Experiment Station of the University of Puerto Rico at Río Piedras and Ensenada.

The mean cane yield differences obtained with the various phosphoric acid applications in each of the experiments described in tables 1 and 2 were not statistically significant. This indicates that the sugarcane yields were not affected by the phosphoric acid applications, even in the case of crop number 3, which was the third crop in succession in which a heavy application of phosphoric acid was tested against no addition of this substance. That is, the phosphoric acid which this soil contained at the beginning of this test sufficed at least for maximum cane yield production of a plant cane and two ratoon crops. Similar statements may be made for the two consecutive crops, number 4 to 11 and 19 to 20.

The lack of response of the sugarcane crop to the phosphoric acid applications in these experiments may have been due to one of two reasons:

1. The soils were in condition to supply the crops with enough phosphoric acid as to render unnecessary and superfluous any further applications of the substance.

2. The phosphoric acid, applied as superphosphate on top of the soil in some of the experiments described in table 1, was not able to penetrate to the root zone and did not exert its possible beneficial action on the crop.

If the first of the two reasons advanced above is applicable, then it must be admitted that sugarcane behaves differently from other crops such as native red beans, eggplant, corn, cucumbers, and sudan grass, all of which have responded markedly to phosphoric acid applications in several ex-

periments performed on soil types similar to those in which the sugarcane has not responded to those applications. The long-time field experiments established at Río Piedras and Ensenada will be continued for a long number of years to determine how long will the minimum 20 pounds P_2O_5 per acre under test be able to maintain crop yields at the same levels as those obtained with the 80 pounds P_2O_5 per acre applications.

TABLE 3

Effect on the sugar cane yield of the method of applying the fertilizer

NUMBER	TREATMENT	F-1017 ON "VEGA BAJA SILTY CLAY," RÍO PIEDRAS. AUGUST 1940 TO MAY 1944			
	Method of applying the fertilizer, (300 lbs. NH₃, 100 lbs. P₂O₅ and 200 lbs. K₂O), per acre per crop)	Plant cane	First ratoon	Second Ratoon	Total for 3 crops
		Tons cane per acre			
1	Phosphate for plant cane and two ratoons mixed with the soil in the furrow before planting; ammonia and potash applied to each crop on two 3-inch deep furrows at the sides of each row one month after last replanting	66.2	50.7	31.7	148.6
2	Phosphate for plant cane and two ratoons mixed with the soil in the furrow before planting, ammonia and potash on top soil for each crop, one month after last re-planting	67.0	53.2	30.5	150.7
3	Complete fertilizer applied to each crop on 3-inch deep furrows at the sides of each row, one month after last replanting	69.6	53.0	31.5	154.1
4	Check—Complete fertilizer applied to each crop, on top of the soil, one month after last replanting	68.4	53.8	34.0	156.2

To test the possibility of the second of the two reasons presented above, a fertilizer placement experiment was performed on a "Vega Baja silty clay" field very near to fields where heavy responses to the phosphoric acid applications had been observed with sudan grass and red beans.

In this experiment, different ways of applying the phosphoric acid were tested. The rates of application, yields obtained and other details are presented in table 3.

The statistical analysis of the results obtained in this experiment indicated that none of the differences between the mean yields corresponding to the various ways of applying the fertilizer was significant. In this case, therefore, the second reason is not applicable.

In view of the above results, it may be concluded for the time being that maximum sugar cane crops may be raised in the coastal plains of Puerto Rico without the application of any phosphoric acid for at least one crop cycle, that is, a plant cane and 2 ratoon crops. The results of the long-time tests under way at present will indicate in the near future whether this period may be lengthened and, if so, by how much.

The above results do not corroborate the statement which appeared in page 84 of the 1927–28 annual report of the Insular Experiment Station of Puerto Rico to the effect that an application of "phosphoric acid in excess of sixty pounds per "cuerda" (0.9712 acre) lowers the gain in yields of sugarcane". As the above data indicate, phosphoric acid applications, up to a maximum limit of four hundred pounds P_2O_5 per acre, have not affected, either for better or worse, the cane yields in the lowlands of Puerto Rico in experiments lasting a maximum of three and a half years. It is believed that the harmful effect of the heavy applications of phosphoric acid, was rather due to the fact that in the treatments involving such phosphoric acid applications the nitrogen level was lower than where smaller applications of phosphoric acid were made.

SUMMARY

1. The yearly investment in phosphoric acid as a fertilizer for sugarcane in Puerto Rico is about $800,000.

2. Phosphoric acid applications have not exerted any effect, either beneficial or detrimental, on the cane yields of several successive sugarcane crops grown on the coastal plains of Puerto Rico.

3. Sugarcane may be raised for several years in the coastal plains of Puerto Rico without any phosphoric acid applications and with no appreciable reduction in sugarcane yields.

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THE EFFECT OF CERTAIN MICRONUTRIENT ELEMENTS ON THE GROWTH AND YIELD OF PINEAPPLE PLANTS

by Francisco J. Ramirez-Silva

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I—INTRODUCTION *

The pineapple was, almost certainly, native of Brazil (5), and very probably the Caribs or Arawaks took some to Puerto Rico before the sixteenth century.

The pineapple (66) is related to the bromeliads and air plants, and it can absorb nutrient constituents through its leaf axils and long-barbed and barbless bayonetlike leaves that protrude from numerous whorls on the main stalk. The so-called pineapple fruit is an aggregate of many individual fruits with their fibrous juice pulp surrounding the core. Pineapple plants are propagated under field conditions from three asexually produced vegetative organs, known respectively as suckers, slips, and crowns. (See figure 1). In some varieties each individual fruit has a number of seeds which can be used for propagation but the most common practice is to plant slips or suckers. The slip resembles a miniature plant and is produced near the base of the fruit. The suckers resemble the slips, but are larger. The crown slip is produced at the top of the fruit; it is rarely used for propagation.

After one crop of pineapples is harvested a second crop, or ratoon, is produced from a new plant resembling a sucker which is formed at the base of the main stalk in contact with the soil.

Pineapple plants respond to good soil that must be well managed, not too alkaline or wet, and well aerated. The acreage in pineapples in Puerto Rico is relatively low compared to that in other crops. The total value of the export crop is high, although very low if compared with sugar cane. Practically all fresh and canned pineapples exported go to the United States where they are considered of good quality and get the highest prices.

The pineapple growers of Puerto Rico claim that the yields from native slips show a yearly lowering in production, as if they were degenerating or as a result of a "rundown" of the stock. Slips imported from Cuba when first planted in Puerto Rican soil, show considerably greater vigor than native slips. They give higher yields than the native stock brought originally from Cuba. It has been observed also that Cuban stock will show signs of decreased vigor and yield after a few years of propagation. Since pineapples are,
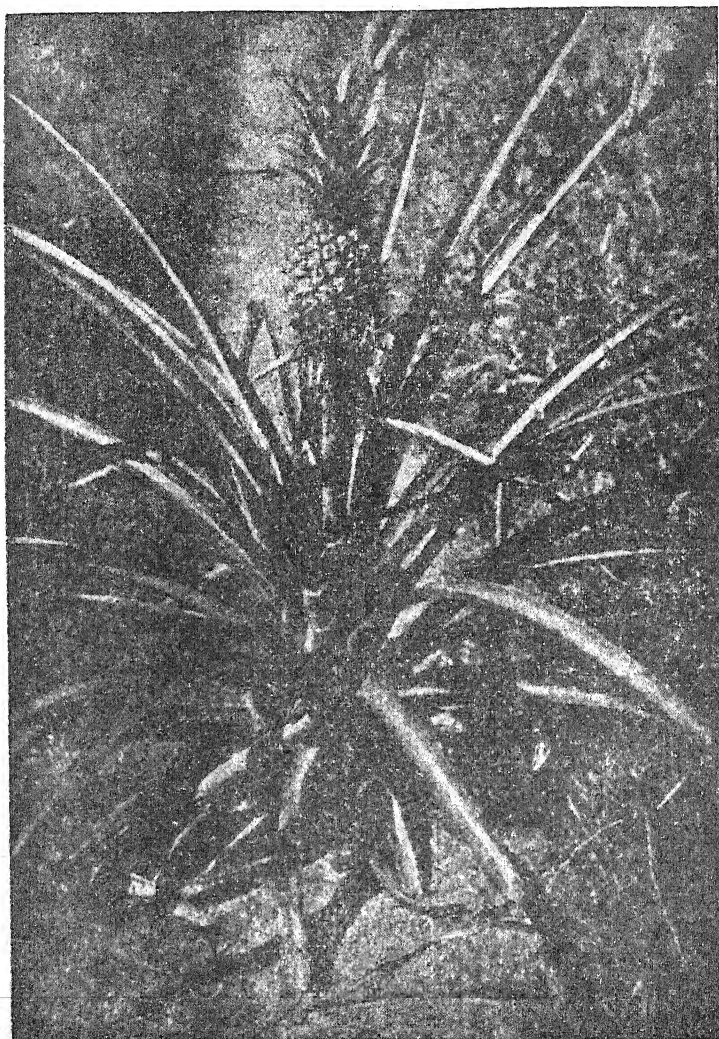
FIGURE 1.—A pineapple plant showing the position of slips, suckers and crown.

as explained above, propagated under field conditions from asexually produced vegetative organs, the degeneration is probably not on a genetic basis. On the other hand, no degenerative disease such as mosaic has ever been found. Hence, there is evidence of the possibility of a nutritional disturbance, perhaps in the micronutrient elements, that may produce vegetative organs with low reserve nutritional elements. These, on being planted in a soil causing such disturbance, may bring about the above-mentioned apparent degeneration of the stock.

This nutritional derangement may have been produced by changes in the soil as a result of faulty cultivation methods, fertilizer practice, constituents of the parent material of the soil, poor crop rotation and conservation. It is claimed, however, that slips planted on virgin soil or on soils not used before for pineapple planting, do not show this degeneration taking place so rapidly as on soils previously used for several years under continuous pineapple production.

Pineapple chlorosis has also been known in Puerto Rico from early times, as shown in works done by Gile (25) since 1911, and by Henricksen (31, 32) in 1925. Similarly, pineapple chlorosis was also known in Hawaii as shown in the experiments of Kelly (50, 51, 52, 53) from 1909 to 1914, also of Wilcox and Kelly (85) in 1912, and of Johnson (45, 46, 47, 48, 49) in 1916–1924.

This yellowing is a source of considerable loss. Spraying of the chlorotic pineapple plants with iron sulfate solution has been practiced in Puerto Rico and in Hawaii, this being an adequate remedy for this malady.

These problems were brought to the attention of Schapelle (67, 68, 69, 70, 71) in 1939, at the Agricultural Experiment Station of the University of Puerto Rico. He set up experiments in order to examine the effect of different nutrient elements under different treatments in the field and under different concentrations of macro and micronutrient elements in solution cultures. His investigations on the nutritional aspect of these pineapple problems in Puerto Rico were followed by the experiments of Hopkins, Pagán, and Ramírez-Silva (40). The experiments presented in this dissertation are part of this series of greenhouse and field investigations on the above-mentioned pineapple problems. The effect of the micronutrient elements: iron, manganese, aluminum, boron, copper and zinc, on pineapple growth and production was examined by means of different treatments in solution cultures.

In accordance with Hoagland (34) who considers the terms "minor elements," "rare elements," and "trace elements" as inappropriate, we use the term he suggested, that is, "micronutrient" elements. Among these are iron, manganese, aluminum, boron, zinc, copper, etc., called micronutrient elements because of the minute concentrations in which they are found in plants. Very minute quantities of them are required to perform their essential functions in plant nutrition. Also, small amounts of them are enough for plants to restore themselves from the specific abnormalities and impaired physiological functions caused by their deficiency in the nutrient medium.

In contrast with these "micronutrient" elements there is a group of elements once called "essential" elements and now usually called "major" elements in plant nutrition. Among them are: nitrogen, potassium, phosphorus, calcium and magnesium. As they are found in greater quantities, they will be referred to as "macronutrient elements." In this sense, the idea of either major or minor, or of essentiality, will not be erroneously conveyed. It may be the case that the so-called "minor elements" be of "major" importance in some cases of plant nutrition. No element is "major" or "minor"; they may be either macronutrient or micronutrient, according to their concentration in plants.

The artificial culture method has been found to be a valuable tool in plant nutrition research. This method has been termed "hydroponic," "water culture," "sand-culture," "gravel-culture," "solution-culture," etc. The term "water-culture" is widely used, but it is not as accurate as the term "solution-culture," since it is a solution of nutrient substances that is used in this artificial method. Of course, when sand or gravel is used in the culture media, the corresponding term of sand or gravel culture is appropriate.

II—REVIEW OF LITERATURE

A. *The growth of plants in artificial media in relation to the study of plant nutrition*

In order to study the role played by nutrient elements in the plant, and in an attempt to separate this from the problem of the availability of the nutrient elements in the soil, that is, from the soil-solution problem, the artificial culture method has come to be a valuable tool in this field of research.

The method of growing plants in nutrient solutions has been used as the best known means of controlling the concentrations, pH,

and proportions of nutrients fed to plants in experimental treatments. The convenience of this method, as well as the objections to it, depend upon the specific problem under experimentation.

Since the earliest recorded experiments with solution cultures by Woodward (61) in 1699, and later on by procedures developed by Sachs, Knopp, and Nobbe, from 1859 to 1865, the modifications introduced by means of different formulas and techniques have made this medium of growth for plants very useful for the purposes of fundamental experimentation and preliminary trials for field work and research.

Hoagland (34), on his discussion of the topic of growth of plants in artificial media in relation to plant nutrition, taking in consideration the dominating phases of this subject, appraises the far-reaching scientific significance of this method and its great service in understanding the nature of the soil-plant system.

According to Miller (61), nutrient solution formulas have been proposed by Tollens in 1882, Schimper in 1890, Pfeffer in 1900, Crone in 1902, Tottingham in 1911, Shive in 1915 (73), Hoagland in 1920, and many others. The osmotic pressure of the solutions has been considered, also, the relationship between proportion of salts, the growth effect of light and temperature, the relative absorption of ions, and the functions of the elements essential to plant life. Miller (61) shows a list of useful formulas for nutrient solutions.

Shive's (73) three-salt solution of KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$ has been the basis of much experimentation by him and others. They have considered the osmotic pressure best fitted to certain crops, the supply of essential elements, and the relation of the elements as found in soil extracts. The formulas derived from Shive's three-salt solution have been classified in series according to osmotic pressures and the molecular proportions of the dissolved components. They are designated by serial numbers when reference is made to them in experimental work.

For experimental purposes it has been recommended that renewal of solutions be made at least every month, and if possible continual removal, aeration by bubbling air, adequate temperatures, uniformity in light supply, and the use of pure chemicals and distilled water.

The works of Lowhwing (54) in 1932, of Clark and Shive (19) in 1932, and of Bryant (14) in 1934, show that nonaerated nutrient solutions tend toward a greater ratio of roots to tops. This suggests the idea of growing plants for a certain period of time, in the beginning without aeration, later on aeration is applied as required for normal crop production.

The preparation of separate solutions to prevent precipitation of concentrated salts upon being mixed was recommended by Tottingham (81) in 1914.

B. *Researches on nutrition of pineapple plants as related to micronutrient elements*

During the last two decades mineral deficiencies in plants have been the subject of much experimentation in the field of plant nutrition. Nevertheless, the question of toxicity due to excess of certain nutrient elements has not been emphasized to a great extent from the standpoint of practical plant culture. The balancing, additive, and antidoting effects between certain nutrient elements have been the subject of still less investigation. Examples of this toxicity are shown by manganese in pineapple plants, as reported from Hawaii by Kelly (50, 51, 52, 53) in 1908–1912, by Johnson (45, 46, 47, 48, 49) in 1916–1924, by Wilcox & Kelly (85) in 1912, and by McGeorge (58) in 1923.

In 1911–1916, Gile (25, 26) and Gile and Ageton (27) found a type of chlorosis of pineapples in Puerto Rican soils that contained large quantities of calcium carbonate and no excessive manganese. They called such chlorosis, lime-induced chlorosis. They noticed that spraying with solutions of iron salts resulted in the restoration of the green color. Johnson (45) in 1916, however, traced the pineapple chlorosis as due to manganese toxicity with a condition of low or no iron absorption, and found that spraying with iron sulphate solution counteracted the effect of high manganese present in the soil. On the other hand, Gile (25) found that this spraying of plants had to be repeated so often that it was not economically practicable.

As we are dealing with the general term "chlorosis", it must be explained here that it stands for a diseased or unhealthy condition of the leaves shown by the loss of the green coloring matter. This yellowing may be due to several factors such as lack of the nutriments producing or intervening, directly or indirectly, with the process of chlorophyll formation, to poor drainage or bacterial effects. After the leaves become strongly chlorotic necrosis ensues, showing extensive black areas.

Schapelle (67, 70, 71) in 1940, attempting to elucidate the problem of the apparent degeneration of pineapple stock and the increasing chlorosis of pineapple plants grown in Puerto Rican soils,

made studies on the effects of macro and micronutrient elements on the growth, yield, chlorosis, and quality of fruits of pineapple plants. He found that potassium was an important factor in the quality of the fruits, that plants responded better to ammonia than to nitrate nitrogen. He noticed that chlorosis was a cause for lower yields. He found profitable the use of frequent spraying with ferrous sulfate solutions. In experiments with pineapples grown in nutrient solutions he used with success a modified form of the solution used by Sideris, Krauss, and Young (74). He had demonstrations of best growth under the conditions of the experiment at a pH around 4.5, that manganese and zinc tended to produce chlorosis, that aluminum and boron tended to counteract the effects of manganese and zinc, and that copper, added at the concentration of 2 p.p.m. controlled a root fungus that caused stunting of the pineapple plants.

After this work of Schapelle (67, 70, 71), Hopkins and co-workers (40) in 1944 started their work along this line of research not only using the pineapple plants but also beans and tomatoes. They found: (a) high amounts of water-soluble manganese in soils from pineapple growing areas, (b) observed the chlorosis of pineapples grown in those soils, and that beans would not grow on them on account of severe chlorosis, (c) that in spite of the correction of chlorosis in pineapples by spraying with ferrous sulphate, certain abnormalities occurred, such as reduction in size of the plant and development of more red pigments than normally, (d) slips imported from Cuba, of the variety "Red Spanish," produced larger and greener plants than slips from the same variety from Puerto Rican plants; but after being planted for two generations in Puerto Rico, plants originally from Cuban slips reverted to the inferior type, (e) many fruits produced in these soils were affected with "short top," that is, a very short crown in the fruit which appears to be brought about by high soluble manganese under high intensity of light. The pineapple soils have pH as low as 3.8 which brings manganese into solution. Tomato plants used for preliminary experiments showed high correlation of maximum growth with the wider ratio of Fe to Mn. Tomato plants show a great sensitivity to manganese, requiring a large ratio of Fe to Mn for normal growth, flowering and production. It was noticed that the reserve of iron in seeds or propagating organs supplied the plant during a certain period of the early growth with the iron necessary to antidote the manganese in the nutrient solution, in either soil or solution culture.

C. *Researches on plant nutrition as related to micronutrient elements used in these experiments*

The micronutrient elements used in this experiment: iron, manganese, copper, zinc, aluminum and boron, have been the subject of an immense amount of research. To review and make a thorough study of all this literature would be outside the scope of this dissertation, but a chronological review of investigations having a bearing on this experiment is necessary. In the following pages such a review is made, taking in consideration that some references have already been mentioned in previous sections.

1. *Iron*

Iron is essential for the growth of plants. In them it is almost universally present in small quantities. Molisch in 1892, as quoted by Miller (61), found that most iron in plants is in a combined, insoluble form. Iron apparently occurs in plants in two forms, namely, the "active and inactive", sometimes also called "available and non-available".

Although plants deprived of iron show marked chlorosis, it has been definitely proved by Willstatter and Stoll in 1913 that iron does not enter into the composition of chlorophyll. Gile and Carrero (28, 29, 30), studied in 1914–1916 the iron requirements of rice, pineapples, and other crops. They noticed that iron, after being transported to the leaves, is immobile, and that colloidal iron is not absorbed by plants. Warburg (83) proposed in 1925 the theory that iron is the oxygen-carrying component of the respiration ferment. Hopkins (36) 1930, believed in the important role played by iron in the cellular processes involving biological oxidation.

According to Miller (61), the researches made by Oddo and Pollaci in 1920, Deuber in 1926, and Pollaci in 1935 show a possible explanation of this role of iron as a catalytic agent in chlorophyll formation, catalyzing the formation of the pyrrole nucleus which is the center of the chlorophyll complex.

Gile and Carrero (30) in 1920, and Willis and Carrero (87) in 1923, agreed in considering that lime–induced chlorosis is due to a depression in the available iron. Rippeal (65) observed in 1923, that chlorosis produced by manganese in the form of soluble salts in solution cultures, was overcome by increasing the supply of iron. He concluded that manganese interfered with the iron within the plant and not with its absorption.

Gericke (24) noticed in 1923 that iron-deficiency symptoms were more acute in plants under lights of high intensity than in lights

of low intensity. This should be examined according to the results of experiments made by Hopkins, Pagán and Ramírez-Silva (40) in 1944. They noticed phototropic effects of manganese in the absence or low level of iron. It is possible that Gericke was taking for iron deficiency what may be called manganese toxicity.

Burk, et al (16), made a report on the experiments made on metallic humates up to the year 1931 and, with reference to iron humate, they pointed out important facts that show the advantages in using humic acid for furnishing iron in a permanently soluble form in soils and in artificial culture media. The humic acid itself does not act by increasing directly the availability of constituents added to or present in the culture medium; or by activating toxic metabolic products; or by affecting surface tension, viscosity, and potential differences between culture medium and organism, or the oxidation potential of the media; but provides iron for growth and nutrition in a more highly available form.

Horner, Burk and Hoover (41) explained in 1934 a method of preparing humate metals from the salts of the corresponding metal and humic acid made from sucrose. This method is simple and provides a soluble form of iron at a very wide range of pH values. It is of great utility in solution cultures where available iron is required for treatments at different pH values, and also when the culture solution is subjected to variations in pH. These humates are stable in alkaline, neutral and moderately acid media, and are not precipitated by phosphates.

The role of iron in plant metabolism should be related to enzymes called iron enzymes, namely, catalase, peroxide, citochromes, indophenol oxidase, and others.

2. *Manganese*

Manganese is widely distributed in nature. It is an essential element for plant growth and functions in the synthesis of chlorophyll and carbon assimilation. According to McHargue (60), previous to 1774 the compounds of this element were confused with those of iron. In that year Scheele discovered that the metal found in pyrolusite, manganese, is altogether different from iron. Gauhn isolated the metal shortly afterwards. Thus Scheele started the work to investigate the functions of manganese in plant economy and its occurrence in soils. Ninety years later Sachs was able to prove that plants assimilate manganese and that it cannot replace the functions of iron in plant growth. By 1894 Bertrand had already determined the chemical composition of the sap of the lac tree and found that

its ash contained 2.5 per cent of manganese. The importance of manganese, in spite of the small content of it in plants, was by that time already recognized.

The works of McHargue, in 1914, showed that manganese and iron play an important role in chlorophyll formation and that this element may be toxic to plants at certain levels of concentration in the nutrient solution.

Sachs in 1865 noticed yellowing and etiolation of leaves in an attempt to substitute manganese for iron. Field observations on pineapples grown in manganiferous soils show that the interior of the fruits have a whitish appearance and usually contain excess acidity. Lime application makes manganese toxicity worse.

In 1908 Fukutome (22), from Tokyo, in his experiments on flax, noticed beneficial action when iron sulfate was added to manganese treatments. This is sometimes termed antagonism, counteracting effect, or antidoting action. Masoni (55) also observed in 1911, in his experiments with corn and lupines, the beneficial counteracting effect of iron on the detrimental action of manganese.

Stocklasa, J., (79) noticed in 1911 that aluminum and manganese have an additive beneficial effect on the growth of several plants.

Wilcox and Kelly (85) in 1912, in their experiments with pineapples, grown in Hawaii, made a thorough study of the physiology and chemistry of pineapples as affected by manganese toxicity. They do not mention the antidoting action of iron. They observed the mechanism of chlorosis, and the bad effects on roots due to excessive soluble manganese in the nutrient solution.

Kelley (53) in 1914 indicated that manganese may have an effect upon soil so as to bring about the mobilization of calcium and magnesium, and that it may stimulate the oxidation going on within the plant and in the soil.

The antidoting action of other cations as Ca, K, Na, and Mg on Mn was observed by McCool (56) in 1913. It may be that the beneficial effect of manganese is only due to the association or counteraction of other cations. He noticed that the deleterious effect of manganese varied inversely with the intensity of light.

Funchess (23) noticed in 1918 that nitrates and nitric acid increased the toxic concentrations of manganese.

Hopkins (36, 37) in 1930 reported the increase of growth of chlorella six hundredfold by addition of manganese to the nutrient solution, and he suggested that manganese functions in an indirect

manner in plants by its action upon the oxidation of iron. Hopkins (39) also noticed slow growth of *Lemna Minor* with iron and no manganese.

Bortner (11)) in 1935 observed chlorosis produced in tobacco plants by manganese in concentration of 15 p.p.m. in the nutrient solution, and noticed the antidoting effect of phosphorus. On the other hand, Sherman and Harmer (72) in 1941 found symptoms of manganese deficiency in oats manifested by specks and chlorosis which were prevented with the application of manganese.

Hopkins, Pagán and Ramírez-Silva (40) in 1944 found increase in growth of beans and tomatoes when manganese in soil was immobolized in the soil, and still more growth if iron humate was added. Marked detrimental phototropic action was noticed with excess supply of manganese, and in this effect, the antidoting action of iron was very effective.

Arnon (3), in his report of a review of the research done in mineral nutrition in plants for the year 1943, presents the following hypothesis offered by Somers and Shive: The cells of plants can tolerate only a certain definite concentration of iron which is of ferrous valence. The function of manganese is to regulate the concentration of ferrous ion. Manganese ions oxidize ferrous to ferric ions which precipitate in the form of "ferric-phosphorus organic complex," rendering iron physiologically inactive.

3. *Copper*

Copper is widely distributed in plants in considerable quantity; but according to research done it has stimulating action only at very low concentration, and it is generally toxic to green plants. The content of copper in pineapple fruits, according to an analysis shown by Beeson (8), is about 8 milligrams per kilogram. McHargue (59) reported in 1925 the copper content in various plants and plant parts as ranging from traces to 46 parts per million.

Plants respond to adequate copper treatments, as explained by Miller (61), showing increase in vigor, yield, quality, and control of chlorosis. He quotes the work of Maquene and Demossy in 1920 where they show that copper is found in greatest abundance in cells that are active in growth, and that its translocation is controlled by metabolic processes.

In the raw peat soils of the Everglades of Florida, Allison, Bryan and Hunter (2) in 1927 found copper to be a specific limiting factor, giving response in growth and production in a remarkable way when 30 and 50 pounds of copper sulphate per acre were applied to the soil.

Miller (61) gives a review of outstanding research done up to 1945 with copper and he points out the beneficial effect in some crops like onions, and in some fruit trees, when used as a fertilizer or as spray.

Hopkins (38) found in 1933 no increase in growth attributable to additions of copper to culture of *Lemna* and *Chlorella.*

Skoog (76) claims that copper may be related to the respiratory process.

The essentiality of copper for many species has been recently demonstrated by Hoagland and others, as reviewed by Petrie (64).

Felix (21) experimented in 1927 with onions and lettuce on the reclaimed muck lands of Central New York and found copper to be a limiting factor. Lack of copper produced a specific anatomical abnormality known as "rabbit ear". Onions fertilized with $Cu\ SO_4$ produced better colored and thicker scales.

It should be noticed that according to the findings of Waddell and Steenbock (82) in 1929, copper is regarded as a necessary adjunct to iron in the regenerated of anemia in animals. This may be considered as parallel to its effects in chlorophyll regeneration. Schappelle (71) claims that copper at a rate of 2 p.p.m. in the nutrient solution controlled a root fungus that caused stunting of the pineapple plant.

The so-called copper enzymes and copper containing proteins should, in part, show the relation of copper to respiration and oxidation processes in plants.

4. *Zinc*

The effect of zinc on plant metabolism is one of the most interesting phases of the field of plant nutrition.

The essentiality of zinc in corn plants was recognized, according to Miller (61), by Maze in 1915, and by Somer (77) in 1928, who noticed abnormalities in the growth of buckwheat and sunflower, and in the flowering of *Vicia faba.*

Bertrand and Andreitcheva (9) in 1933 considered the zinc content correlated with a high chlorophyll content.

According to Miller (61) zinc deficiency caused the plant disease called "little leaf," as demonstrated by works of Chandler, Hoagland and Hilbard in 1933, on peaches, apricots, tobacco, squash, corn, mustard, tomatoes and other plants.

Mowry and Camp (62) in 1934 found that spraying with zinc sulphate, or its addition to soil, made tung trees recover from bronzing.

Chapman, Vanselow and Liebig (18) produced mottle leaf by omitting zinc from culture solutions. The "mottle leaf" disease in citrus orchards is caused undoubtedly by zinc deficiency. Hoagland (34) describes the fight against this disease in California during twenty years, and says that not a good clue to its cause has been found yet.

Spraying with appropriate zinc compounds, he says, is effective and commercially practical. In Florida, the disease on pecans called "rossette," in Australia a disease of pine trees, and in Hawaii pineapples showing distorted blades, are conditions remedied by zinc sprays. It has been observed that for various reasons zinc in the soil is sometimes not made available to plants. It might be fixed to soil colloids. Certain soil organisms are a recognized factor in the non-availability of zinc by competing with the plant. Some plants have more capability than others for absorbing zinc when it is in a low supply from nutrients. Certain plants like alfalfa show a high ability to absorb zinc from the nutrient media.

Hoagland (34) considers that the quantitative requirement of zinc, as well as the deficiency symptoms, are in part governed by climatic or seasonal factors. High intensity of light aggravates the zinc-deficiency symptoms. (This is an important factor in tropical agriculture.) According to Hoagland (34), and the work of Skroog (76) about this phototropic action of zinc, auxin formation in plants is connected with zinc nutrition. Auxin breakdown is promoted by short-wave light.

The translocation of zinc is effected through the breakdown of the zinc protein compounds under the action of reduced light, thus releasing the zinc which is transplanted to regions of active growth. So, zinc is directly or indirectly connected with protein synthesis in plants. As it does not undergo reversible valence changes, its action in oxydation-reduction systems, if any, must be an indirect one, or due to its influence upon oxidizing enzymes and its interrelation with iron. Hence, zinc is related to respiratory processes and the maintenance of normal concentration of auxin in tissues as claimed by Skoog (76).

Thatcher (80) believed that copper and zinc are mutually counterbalancing catalyzers for hydrogen exchange, as shown by their strikingly opposite effect upon reversible oxidation-reduction reactions of both glutathione and ascorbic acid.

Leaf chlorosis in grape fruit trees in Puerto Rico, resembling the "mottle-leaf" in California and the "frenching" in Florida, was successfully controlled by Jensen (44), at the Federal Agricultural

Experiment Station of Puerto Rico, by spraying with zinc sulfate solution. Pineapple, under certain conditions of zinc deficiency, according to Nightingale (63) in 1942, show characteristic spots or blisters from which they recover by spraying with zinc sulfate solution.

As quoted by Beeson (8), a pineapple fruit analysis showed 20 milligrams of zinc per kilogram. Willis (86) showed that during the last fifteen years experimentation on this micronutrient element has demonstrated the essentiality of it for the normal growth of green plants, and its deficiency as causing characteristic chlorosis, mottle leaf or frenching, and "rossete" or "little leaf" in fruit trees.

5. *Aluminum*

Aluminum is very abundant in soils. It has been found in all plants that have been analyzed but, as a rule, the percentage of aluminum in plants is very low. It may thus be considered a micronutrient element. Grains and vegetables analyzed by Meyers and Voegtlin, as quoted by Miller (61), contained from 0.045 per cent dry basis in wheat flowers, up to 0.996 per cent in cotton seed. As to the role of this element in the growth and production of plants, Miller (61) reviewed the works of Yamano in 1905, who found injurious effects caused by 0.2 per cent ammonium alum on wheat and rye grown in nutrient solutions, and 0.8 per cent to be a fatal dose. Prianishnikov, in 1911, grew wheat, oats, barley, peas and buckwheat in sand cultures fertilized with aluminum phosphate and calcium carbonate alone. Baguley found in 1912 the iron and aluminum phosphate combination to be better. Kratzman observed in 1914 toxic effects of 0.005 per cent concentration of aluminum salts. Others, in experiments done after these, have found toxicity of aluminum salts at certain low levels of concentration in the nutrient media.

The soil-plant aspect of aluminum has been studied more than others. In fact, considering soil work, aluminum shows a great complexity in relation to other elements, it being one of the principal components of soils and soil colloids. Its availability is greater at lower pH.

Schapelle (71) showed beneficial action and recovery from injuries brought to pineapple plants grown in nutrient solutions lacking in aluminum. But Abbot (1) found in 1913 aluminum to be a toxic agent in the marsh regions of peaty sand, and also in culture solutions.

Barnette (6) in 1923, using solution cultures and upon observing the toxic effects of aluminum ions, determined that such toxicity was not due to acidity per se, but to the hydrolysis of aluminum salts.

The function of aluminum within the cell of the plant is ignored yet. Miller (61), reviewing the work of Fluri in 1907, mentioned the consideration that aluminum has an indirect effect in starch disappearance from the cell by increasing protoplasm permeability, diastatic action, and slowing photosynthesis.

Stocklassa (79) found in 1911 that aluminum and manganese together stimulated growth of several species of plants.

Blair (10) observed in 1923 the detrimental effect of soluble aluminum in soils upon roots. McGeorge (57) reported in 1925 that he noticed toxidity of aluminum on roots, in culture solutions, at the pH of acid soils. Haas (35), nevertheless, observed beneficial effect of aluminum in solution cultures of lemon, leafy-twig cutting, when a good supply of phosphorus is present.

Burgess (15) determined, 1923, the availability of aluminum in some soils. At pH 4 to 5 he found 388 parts per million while at pH 5 to 5.8 it was lowered to 36 parts per million.

Arnon (3), in a review on plant nutrition for the year 1945, says that Liebig, Vanselow and Chapman claim that they found that aluminum at low concentrations counteracts copper toxicity in citrus grown in culture solutions. It seems, according to them, that the case of the beneficial action of aluminum depends on its action against the toxicity of copper.

At high concentrations (2.5 to 5 p.p.m.) aluminum gave a curious stimulation of root growth accompanied by depression of top growth. In the absence of aluminum, excessive copper caused a brownish appearance in citrus roots and short swollen laterals which gave the roots a dwarfed, knotty, and unhealthy appearance. Top growth often exhibited iron chlorosis.

6. Boron

Boron is the micronutrient element that has received the greatest attention; and still the mechanism of the function of this element in plant growth is hidden to us. As claimed by Chapman (17), up to now we have not passed beyond the knowledge of the effects of its deficiency upon the meristematic tissue, and its interrelation with calcium. There is a marked similarity between the symptoms of calcium and boron deficiencies. Boron is widespread in the plant kingdom, it probably occurs in all green plants. Since 1857 its

presence was detected in plants by Whittstein and Apogier (88), followed by Baumert (7) in 1888, Hotter (42) in 1890, and Jay (43) in 1895, who analyzed various plants and believed in the universality of boron in the plant kingdom.

The study of the influence of boron upon plant growth called the attention of many investigators. Sand, solution, and soil-culture experiments were made by Augulhon (4) in 1910 and by Brenchley (12) in 1914. Their findings point toward the beneficial effect of boron when supplied to the plant in the right amount. The work of Warington (84) in 1923 on boron compounds on beans, in solution cultures and field experiments, was the beginning of the consideration of the mechanism of boron nutrition in plants. She pointed to the catalytic action and its effect on meristematic tissues.

The effect of boron nutrition on nodule formation in leguminous plants was studied by Brenchley and Thornton (13) in 1925. They found beneficial action on the production of nodules as due to the anatomical conditions of the plants with good boron nutrition. From there on, the boron requirements for nutrition of many crops have been studied, as well as the symptoms of deficiency and toxicity for different plants.

Miller (61) reviews the studies made on boron deficiency, and from the works of Warington in 1926, points out that disintegration of phloem and ground parenchyma, poor development of the xylem, and hypertrophy, discoloration, and disintegration of cambial cells occur when boron is omitted from the nutrient solution. Growth is arrested in the meristematic tissues of root tips, as found by experiments of Sommer and Sorokin in 1928. The effects of boron deficiency in tomato plants, "the guinea pig of the plant nutritionist," are very noticeable: death of terminal growing points of stem, characteristic brittleness of the stem and petiole, and poor brownish roots.

That the function of boron cannot be performed by other elements was found by Warington in 1927. She tried fifty-two other elements. Sugar beet and alfalfa are plants very much affected by lack of boron, and show specific deficiency symptoms. Boron, indeed, will show harm on plants when supplied in excess, bringing about chlorosis. This is probably due to its action against the solubility of iron, as claimed by Rodríguez in 1935. (66ᵃ) An effect of boron toxicity is stimulation of undifferential cell division causing abnormal growth in the regions of its maximun effect.

The essentiality of boron for higher plants is no longer open to dispute. Brenchley (12) and Warington (84) proved that boron

is absolutely indispensable for satisfactory growth of many crops. Both report on the retardation of the development of meristem tissues and discoloration of the stem in plants as specific symptoms of boron deficiency.

Eaton (20) made in 1944 careful observations on the nutritional effects of boron, and noticed that in most plants it accumulates in soluble but largely immobile form.   He suggests that boron becomes attached to some large molecule which, though soluble, is unable to pass through the plasma membrane of the mesophyll cells.   Owing to this immobility of boron in leaf tissue, plants may show symptoms of boron excess in old leaves, and yet not be supplied with excess.   Thus, there may be an overlapping of beneficial and toxic effects in the same plant.   High-light intensity may be responsible for its immobility in the leaves.   Boron deficiency is aggravated by increase of calcium in the nutrient solution, but its toxicity is lowered.   Variations in potassium concentration affect indirectly boron deficiency and toxicity due to the effect of potassium on calcium absorption.

III — OBJECT OF THE WORK

It is the object of these experiments to study:

1. The effect of iron, manganese, zinc, copper, aluminum and boron on the growth and production of pineapples.

2. The antidoting effect of iron on manganese toxicity.

3. The mutual action of these micronutrient elements, and their deficiency or toxicity as affecting root growth, leaves, flowering, fruiting, yield, and quality of the crops.

4. The causative agents of pineapple chlorosis.

5. To verify the data already obtained in other experimentation in this field.

6. To guide future experimentation with pineapple plants along this line.

7. To suggest possible methods to remedy injuries on the pineapple plants as caused by malnutrition of the plant.

IV — EXPERIMENTAL

A. GENERAL PLAN

Solution-culture methods were used in this experiment with a formula of macronutrient elements already found to be good for growing pineapple plants. The facilities of the greenhouse and hydroponic equipment of the Agriculture Experiment Station of the University of Puerto Rico (Fig. 2 and 3) as designed by Schapelle (71) were used. These experiments are a part of a research project of this Station.

The experiments consisted of two series of treatments. One series had nine treatments of micronutrient elements, to study the individual effect of the micronutrient elements when added to the culture solution. Treatment number one had no micronutrient elements added. This treatment showed the combined effect of all the reserve micronutrient elements in the planted slips. It served as a check on the other treatments. Treatments 2, 3, 4, 5, 6, and 7 corresponded, respectively, to additions of iron, manganese, boron, copper, zinc, and aluminum as the only micronutrient elements added to the nutrient solutions. These showed the beneficial effect of the presence of these elements, or their toxic effect, when acting independently at the concentrations added, as compared with treatment 1, to which no micronutrient elements were added. The eighth treatment contained all the micronutrient elements in the concentrations used in the previous treatments. This treatment was another check on the other six treatments. Treatment 9 was the same as 8 except that copper was not added. This showed the effect of lack of copper and was intended to check the results on root injury shown by lack of copper in the experiments of Schapelle (71). This treatment may be used to examine the effect of copper added in treatment 5 and, from this, infer its effect shown in treatment 8 where all micronutrient elements were added, and those of the following series.

Treatments 1 to 9 were designated as follows:

Treatment 1 as –ME (no micronutrient elements added)
Treatment 2 as Fe (iron added)
Treatment 3 as Mn (manganese added)
Treatment 4 as B (boron added)
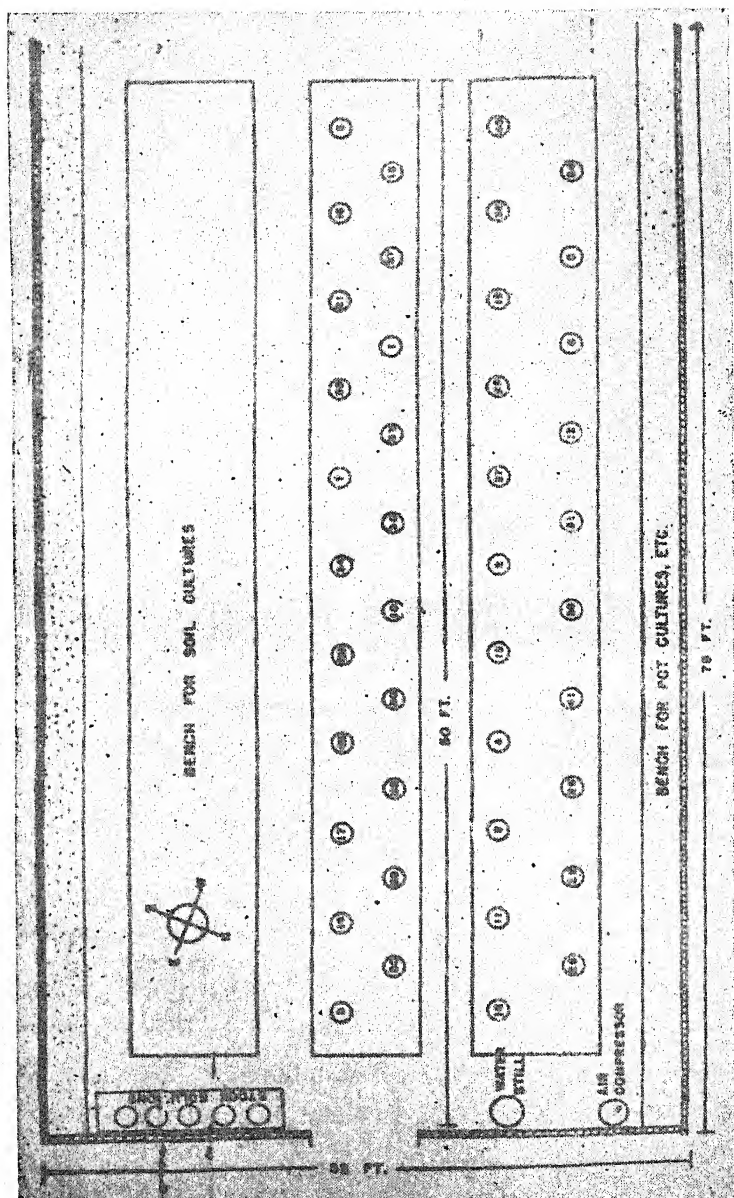Treatment 5 as Cu (copper added)

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FIGURE 2.—Floor plan of the greenhouse showing the arrangement of the solution cultures. The numbers and position of the pineaple plants are shown within the circles.

Treatment 6 as Zn (zinc added)
Treatment 7 as Al (aluminum added)
Treatment 8 as ME (all micronutrient elements added)
Treatment 9 as —Cu (all micronutrient elements minus copper)

The series of treatments 10 to 14 was intended to study the antidoting action of iron against the chlorosis-producing effect of manganese. For this purpose 5 p.p.m. of manganese were added to each one of the five treatments, and a different level of concentration of available iron was added in each. So, treatment 10 had no iron added, number 11 had one p.p.m. added, number 12 had three p.p.m., number 13 had five p.p.m., and number 14 ten p.p.m. All these treatments were supplied with 2 p.p.m. of copper to prevent root injury as reported by Schapelle (71). The micronutrient elements: boron, zinc, and aluminum, were added also, in a concentration of one half p.p.m. each, in order to prevent deficiency of these elements. Treatment 2 with 5 p.p.m. Fe added as the only micronutrient element may also be considered as a member of this series for the purpose of the study of the antidoting effect of iron. The study of the results of the treatments 1, 3, 4, 5, 6, 7, 8, and 9 will throw light on the study of the series of treatments 10 to 14 and viceversa.

The effect of aeration of the culture solution was studied. During the initial period of growth the solutions were not aerated.

For the purpose of this study, observations and data on roots, plant growth, chlorosis, flowering, fruiting, yield, and quality of fruit were taken.

B. Methods

1. *Nutrient solutions*

Pineapple slips of the variety "Smooth Cayenne," 12 inches long and selected for uniformity, were "planted" in culture solutions. All the treatments contained the following concentration of macronutrient elements:

SALT	Grams per liter
$K H_2 PO_4$	0.1316
$Mg SO_4 7H_2O$	0.4100
$Ca(NO_3)_2 4H_2O$	0.4720
$NH_4 NO_3$	0.1260
K_2SO_4	0.1657

which furnish:

112.1   parts per million of **K**
30.0   parts per million of **P**
40.5   parts per million of **Mg**
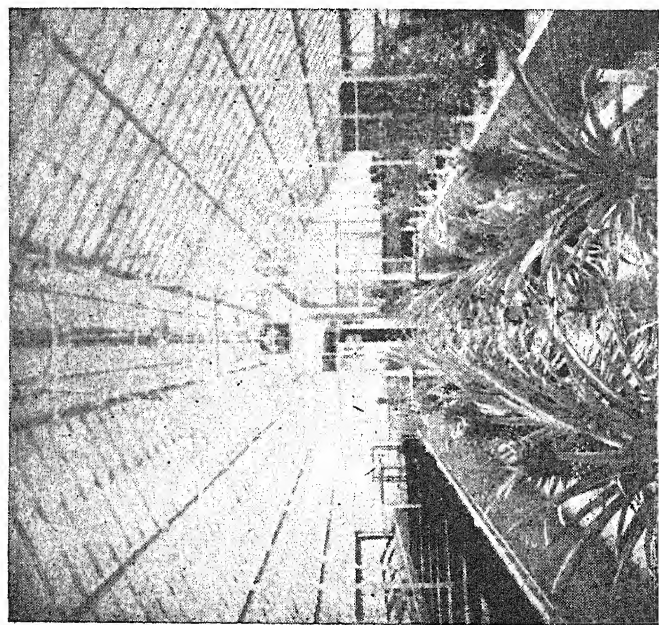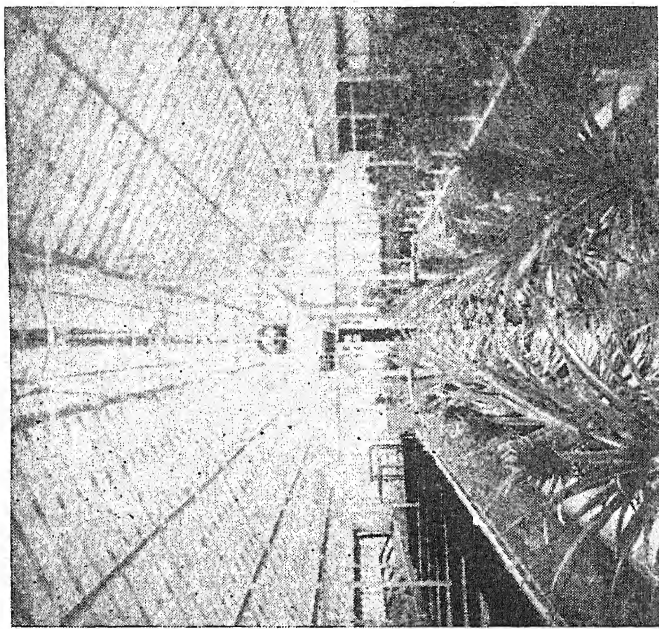80.1   parts per million of **Ca**

FIGURE 3.—Photographic views of the Greenhouse and the Solution Culture Experiment.   Pineapple Plants, 493 days after planting.   The air supply system for aerating the solution is shown.

83.6 parts per million of S
77.9 parts per million of Nitrate N
22.0 parts per million of Ammonia N

The following concentrations of micronutrient elements were added to the different treatments:

TABLE No. I

TREATMENT			PARTS PER MILLION					
No.	Elements	Jar Number	Mn	Cu	Al	B	Zn	Fe as FeSO₄
1.........	-All M. E.....	1- 2- 3.........						
2.........	Fe...........	4- 5- 6.........						5
3.........	Mn..........	7- 8- 9.........	2					
4.........	B...........	10-11-12.........				1		
5.........	Cu..........	13-14-15.........		2				
6.........	Zn..........	16-17-18.........					2	
7.........	Al..........	19-20-21.........			1			
8.........	All M. E.....	22-23-24.........	2	2	1	1	2	5
9.........	-Cu..........	25-26-27.........	2		1	1	2	5

THE IRON-MANGANESE SERIES

No.	Elements	Jar Number	Mn	Cu	Al	B	Zn	Fe as humate
10.........	5 ppm Mn....	28-29-30...........	5	2	¼	½	½	0
11.........	5 ppm Mn Fe 1 ppm.	31-32-33...........	5	2	¼	½	½	1
12.........	5 ppm Mn Fe 3 ppm.	34-35-36...........	5	2	¼	½	½	3
13.........	5 ppm Mn Fe 5 ppm.	37-38-39...........	5	2	¼	½	½	5
14.........	5 ppm Mn. Fe 10 ppm.	40-41-42...........	5	2	½	½	½	10

The solutions of micronutrient elements were prepared as follows:

Element	Parts per Million of the Element	Salt used C. P.	Grams of Salt per Liter
Mn..............	2...................	Mn SO₄ H₂O.......................	0.00615
Cu..............	2...................	Cu SO₄ 5H₂O.......................	0.00785
Al..............	1...................	Al₂ (SO₄)₃ 18H₂O..................	0.012355
B...............	1...................	K₂B₄O₇ 5H₂O.......................	0.007476
Zn..............	2...................	Zn SO₄ 7H₂O.......................	0.008796
Fe..............	5...................	Fe SO₄ 7H₂O.......................	0.02489

In every case stock solutions were prepared, so as to furnish the requisite amount of micronutrient elements, by using a definite adequate aliquot for the corresponding jar used in each treatment. The solution of ferrous sulphate was prepared in small quantities, at the required moment, to be used immediately. It was slightly acidified with sulfuric acid to prevent hydrolysis. Distilled water and C.P. chemicals were used always.

The iron humate added to treatments 10 to 14 was prepared according to the method of Horner, Burk and Hoover (41), and taking into consideration the recommendations of Burk, Lineweaver, Horner and Allison (16).

The macronutrient formula used in these experiments is a modified form of the solution used by Sideris, Krauss, and Young (74). It was used successfully by Schapelle (70, 71) in his work with pineapple plants.

The pH values of the nutrient solutions were determined periodically, by means of a Leeds and Northrup Universal glass electrode potentiometer. A pH value near to 4.5 was maintained by adding the required amounts of a normal H_2SO_4 solution or a dilute solution of $Ca(OH)_2$, according to the change in pH of the nutrient solutions. According to Schapelle (71), pH 4.5 is the optimum value for growth of pineapple plants in culture solutions.

2. *Method of growing the pineapple plants.*

The pineapple slips selected for these experiments were planted in quart culture jars of good-grade white glass which were prepared with suitable wooden lids with a hole in the center big enough to hold the slips. Three cultures were planted for each of the fourteen treatments, so, there were forty-two culture jars. These were distributed at random in the greenhouse. The arrangement and number of the treatments are shown in figure 2. Two photographic views of the experiments, when the plants were already fruiting, are shown in figure 3. The jars were buried in sand to exclude light rays from the solutions.

Table I shows the concentrations of micronutrient elements used for each treatment and the jars numbers.

The macronutrient solution used was the same for all the treatments. Its concentration is shown on page 216. The solutions were changed every month. The concentration of the solution of macronutrient elements was reduced to one half from the 214 day on.

On the 84 day after the slips were planted, the plants were transferred from the quart jars to wide-mouth gallon jars because the root system had grown already too big for the quart jars. For the same reason they were again transferred to 17–liter pyrex jars on the 214 day. The lids of these jars were made of wood and the center holes were lined with cork rings to hold the plants in place.

The roots were carefully drained of the residual solution in them every time that the solutions were changed. To change the solution, the plant was removed together with the lid and the jar was emptied

with a siphon. It was then cleaned of any solid residue and of the old solution. Then the jar was filled to about one half its volume with water. The corresponding aliquots of the stock solution of nutrient elements were added independently one by one, dissolving each one after added. The volume was completed to the 17–liter mark, and the plant put back to its place. The solution was brought to the 17–liter level with distilled water every day.

While the plants were growing in the quart jars, that is, during the first 83 days, the solutions were not aerated. From the 84 day on air was bubbled continuously through the solution. The air bubbles were passed at a uniform rate through all the jars, as exactly as possible. An automatically-pressure-controlled electric air compressor furnished the supply of air. Glass tubing was used to pass the air from the main air pipe into the solutions (see Figure 3).

The greenhouse, where this experiment was set up, was well ventilated by means of an electric fan located near the roof at one end of the building. (See Figure 3).

3. *Observations and data to be obtained.*

The plants were observed every day to note: (*a*) any change in conditions of growth, (*b*) root volume and development, (*c*) date of appearance and magnitude of chlorosis, (*d*) necrosis in leaves, (*e*) intensity of greenness, (*f*) condition and pH of solutions, (*g*) date of the beginning of blooming and of attainment of mature fruit, (h) weight and size of fruit and crown when harvested, (*i*) analysis of the juice of the fruit ripened out of the plant, for reducing sugars, total sugars, total acidity and density.

4. *Chemical methods of analysis.*
(*a*) *Extraction of juice*

The ripe pineapple fruits were carefully peeled from the non-edible rind, and the whole fruit cut in pieces and subjected to 500 pounds pressure per square inch in a Carver laboratory press. The juice expressed from the whole pineapple fruit was collected for analysis.

(*b*) *Density determination*

The degree brix of the juice was determined using brix hydrometers calibrated at 20°C. The readings made were corrected to 20°C, using the scale for corrections attached to the thermometer within the body of the hydrometer.

(c) Determination of reducing sugars

The official method (75) for the determination of reducing sugars and total sugars was used, that is, Lane–Eynon general volumetric method, as described in section XXXIV 32 of the Official and Tentative Methods of Analysis (75). This method uses Soxhlet's modification of Fehling's solution, as reagent. Solution (A) consists of 34.639 grams copper sulfate (CuSO$_4$ 5H$_2$O) dissolved in distilled water and diluted to 500 ml., filtered through prepared asbestos in a Cooch crucible. Solution (B) is the alkaline tartrate solution, and is made by dissolving 173 grams of Rochelle Salts and 50 grams of sodium hydroxide in distilled water and diluted to 500 ml., then allowed to stand for two days and filtered through prepared asbestos. This solution was standardized according to the procedure in Section XXXIV, 33 of the Official Methods of Analysis (75), taking 5 ml. of solution (A) and 5 ml. of solution (B).

The determinations were made on a portion of the freshly extracted juice diluted 1 to 10. A one-per-cent-aqueous solution of methylene blue was used as the internal indicator. A preliminary incremental titration was made on the first sample, and the determinations made in triplicate, according to Section XXXIV, 34 (75), following a similar procedure to the one used for standardization of the reagent.

The calculation of the reducing sugars was made using Table XV Section XLIII of the Official Methods (75), taking the factors in the column for solutions having one gram of sucrose in 100 ml. of the juice solution used for titration. Of course, the readings of the volume in ml. of solution required to react to the end-point with the amount of reagent used, was divided by the concentration factor of the reagent's solution, to obtain the ml. that would be required for a reagent with a concentration factor of 1.0.

As the values thus obtained give the grams of reducing sugar in 100 ml. of the solution of juice used in the titration, multiplying these weights by ten will give the grams of reducing sugar in 100 ml. of juice. To obtain the percentage of reducing sugars weight in the juice, the weight in grams of reducing sugars in 100 ml. of juice is divided by the specific gravity 20°/4°C of the juice as determined from the brix reading, from Spencer (78).

(d) Determination of Total Sugars

Total sugars were determined promoting total inversion by adding 2 ml. of concentrated HCl (specific gravity 1,1029 at 20°/4°C)

to 10 ml. of the freshly extracted juice in a 100 ml. volumetric flask, left overnight for total inversion. Then the solution was neutralized with NaOH and brought up to the 100 ml. volume. From this, 10 ml. were diluted to 50 ml. and analyzed for reducing sugar by the same method given above for the fresh uninverted juice. The weight in grams of invert sugar in 100 ml. of the titrated solution of juice as calculated from the factors of Table XV, in the Official Methods (75) on the first column for no sucrose, must be multiplied by 50 to account for the two dilutions made: 10 to 100 and then 10 to 50. This gives the weight in grams of total sugars as invert sugar in 100 ml. of fresh juice. This value divided by the specify gravity $20°/4°C$ gives the percentage by weight of total sugars, as invert sugar in the juice.

(e) *Total Acidity*

The total acidity was determined by titrating the freshly extracted juice against a standard NaOH solution, taking 10 ml. of the juice. Phenolphthalein was used as the indicator.

According to E. K. Nelson of the Bureau of Chemistry, U.S.D.A., quoted by Hericksen (33), citric acid constitutes about 87 per cent of the total acids of pineapple juice. The acidity of the juice is calculated on the basis of per cent of citric acid in juice. This is done by multiplying the normality of the acid in the juice by one tenth of the gram equivalent weight of citric acid, that is, 6.4, and dividing by the specific gravity $20°/4°C$ of the juice so as to reduce it to gravimetric basis.

C. *Results and Discussion of the Effect of Micronutrient Elements.*
  1. *Chlorosis*

The observations on the chlorosis shown by the plants, are expressed numerically in table II, indicating the total severity of chlorosis per treatment of three plants, the day of appearance, and of any change in the severity.

The numerical evaluation has been made on the following scale of values per plant:

Slight chlorosis	1.0
Medium chlorosis	2.0
Strong chlorosis	3.0
Very strong chlorosis	4.0
Necrosis	5.0
Very strong necrosis	6.0
Death of each plant	8.0

TABLE No. II

OBSERVATIONS ON CHLOROSIS OF PLANTS EXPRESSED NUMERICALLY FOR THREE PLANTS

Number	Treatment	Days after planting													Weighted averages 414 days
		88	90	94	97	101	107	114	119	132	143	170	214	414	
1	-M. E.	1				1	2	2	3	4	5	6	6	6	4.2
2	Fe.														0
3	Mn.		1	2	2	3	5	6	6	6	10	10	10	10	7.1
4	R.			1	1	2	2	3	3	4	5	8	8	8	5.3
5	Cu.					2	3	3	6	9	14	11	14	11	9.2
6	Zn.					1	1	2	2	4	4	5	5	5	3.5
7	Al.			1	1	1	2	2	3	3	3	4	4	4	2.6
8	M. E.														0
9	-Cu											1	1	1	0.6

IRON-MANGANESE SERIES—5 PPM OF MANGANESE

Number	Treatment	Days after planting													Weighted averages 414 days
		88	90	94	97	101	107	114	119	132	143	170	214	414	
10	Fe 0 ppm	3	3	6	9	12	12	12	12	12	16	18	18	18	13.0
11	Fe 1 ppm														0
12	Fe 3 ppm														0
13	Fe 5 ppm														0
14	Fe 10 ppm														0

BASIS OF NUMERICAL EVALUATION PER PLANT

slight chlorosis	1.0	very strong chlorosis	4.0	
medium chlorosis	2.0	necrosis	5.0	
strong chlorosis	3.0	strong necrosis	6.0	
		dead plant	8.0	

See Table I for concentration of micronutrient elements in each treatment.

For the purpose of comparison on the chlorosis produced by each treatment, averages have been calculated as "weighted averages". The magnitude of the severity is multiplied by its duration in days, and each summation of these products is divided by 414, that is, the number of days from the time of planting to the time of blooming of the plants.

The following method was used, taking treatment three as an illustration.

According to table II, in this treatment chlorosis appeared on the 88 day.

                The chlorosis value 1 lasted   6 days
                The chlorosis value 2 lasted   7 days
                The chlorosis value 3 lasted   6 days
                The chlorosis value 5 lasted   7 days
                The chlorosis value 6 lasted 29 days
                The chlorosis value 10 lasted 271 days

Multiplying each value of intensity of chlorosis by the number of days it lasted, then this gives:

                    Without chlorosis 88 days
            1 multiplied by   6 equals   6
            2 multiplied by   7 equals   14
            3 multiplied by   6 equals   18
            5 multiplied by   7 equals   35
            6 multiplied by   29 equals 174
           10 multiplied by 271 equals 2710
                                       ─────
            Total "chlorosis days"   2957
            Total number of days 414
            Average per day (three plants)   7.1

The average is calculated on the total growing period of 414 days. Some treatments showed chlorosis before others. Averaging for the total period of growth takes this into account.

Apart from the information given in table II, the following observations were made:

  a. Treatments 2, 8, 11, 12, 13, 14, that is, all the treatments containing iron, showed plants with a good green color, especially treatment 2 that had iron as the only micronutrient element added.

  b. The three plants of each treatment showing chlorosis were not affected equally in each case, some showed very slight chlorosis too low to be evaluated. The variations in reserve iron in the planted slip may be the chief reason

for this. The table gives information about the starting date, intensity in each case, and the rate of increase.

c. One plant in treatment 10 never bloomed and finally died of an extremely strong necrosis.

d. One of the plants of (copper) treatment number 5, showed also strong necrosis but was able to survive and bear fruit.

e. Attention should be given to the fact that treatment 3 had only two parts per million of manganese, while number 10 had five parts per million and the other micronutrient elements.

f. The degree of greenness in treatments 11 to 14 increased with the increase in iron in these treatments, but was not as high a green color as in number 2 that had iron as the only micronutrient element added. (Treatments 10 to 14 had other micronutrient elements added besides the manganese and iron. See Experimental Methods).

Discussion of the results on the chlorosis produced

All the treatments with iron added: 2, 8, 9, 11, 12, 13 and 14, were exempt from chlorosis.

Treatment 2, with iron as the only element added at 5 ppm, was the greenest of all treatments. Treatment 1, with no elements added, showed chlorosis; so, the iron added in treatment 2 was enough to prevent the chlorosis caused by chlorosis-producing elements in reserve in the planted slip. In other words, the reserve iron in the slip was not sufficient to counteract the chlorosis-producing tendency of the other elements through the whole period of growth. Extra nutrition of iron was required, as shown in treatment 2, to prevent chlorosis. Treatment 2 was greener than treatment 8 where the other elements were added and in which still no chlorosis was produced.

Treatments 10, 11, 12, 13, and 14 of the iron-manganese series, showed more definitely the counteracting action of iron against the chlorosis-producing action of manganese, and possibly of the other elements added in this treatment. While treatment 10 showed marked necrosis (one plant could not survive); treatment 11 (only one part per million of iron added) produced green chlorosis-free plants. Treatment 10 had five parts per million of manganese added and other micronutrients in smaller concentrations. (See Experimental Methods.) As will be shown later, these other elements have some tendency to produce chlorosis, especially copper.

Figure 4 shows plants under treatments 10 and 11, on the 102 day after planting. Notice that the plant of treatment 10 was strongly chlorotic, and that of treatment 11 was normal. Figure 5
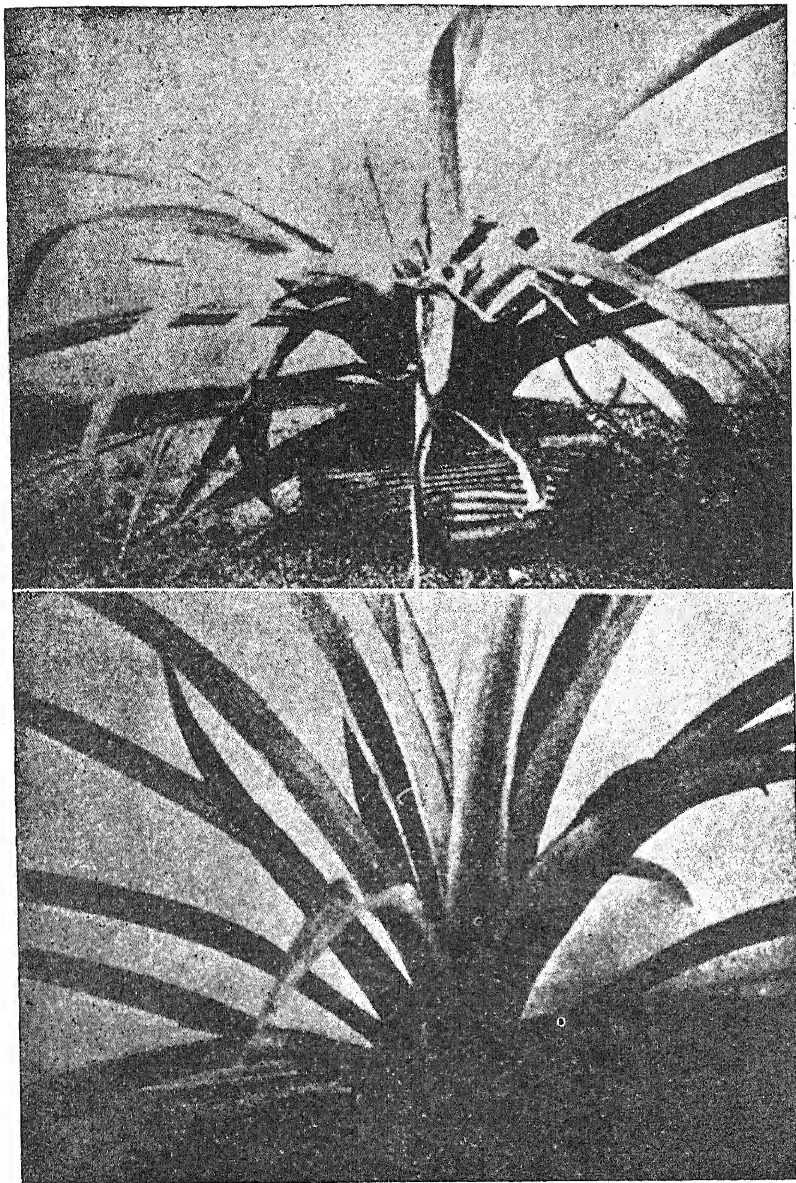
FIGURE 4.—Photographs of plants, 102 days after planting.
Treatment 10, above. Micronutrient elements added to the culture solution:
5 ppm of Mn, 2 ppm of Cu, ½ ppm of B, ½ ppm of Zn, and ½ ppm of
Aluminum. The plant is strongly chlorotic and necrotic.
Treatment 11, below. The same micronutrient elements as above but with
one ppm of iron added. The plant is growing normally.

shows plants of the same treatments 493 days after planting. The plant of treatment 10 was almost dead and that of number 11 produced a normal fruit with a beautiful crown, a good sign of vigor and good quality.

The appearance of treatments 11, 12, 13, and 14, showed an increase in greenness with the increase of iron. No detrimental effect due to iron was noticed in any one of the treatments where iron was added.

The chlorosis produced by toxic concentrations of iron mentioned by Arnon (3), and the necessity of certain concentration of manganese to bring about a balance of the ferrous and ferric ions for proper chlorophyll formation, are not shown in these treatments. It may be that in the case of treatment 2, where iron was the only element added in five parts per million, the concentration was low enough, or the reserve manganese in the slip was at the right level; but in any case, there were no signs of iron toxicity. There are good reasons to believe that the toxicity of iron, if any, would be at a very high level in pineapple plants.

Treatment 5, that had copper as the only minor element added, showed strong chlorosis and even necrosis. This points out the conclusion that copper produces chlorosis at certain levels of concentration in the nutrient solution, if in the absence of iron. These results show the action of copper stronger even than that of manganese, which in treatments 3 and 10 induced chlorosis very markedly and in proportion to its concentration in the nutrient solution. See figure 6.

Boron also produces chlorosis when added to the nutrient solution in the absence of iron, or in the presence of insufficient iron to antidote its action. Compare treatment 4 with 1.

The above conclusions can be shown numerically as follows:

The average of the "weighted averages" of chlorosis of all treatments from 1 to 9 having iron, i.e., treatments 2,8,9, is 0.2; while the average for all treatments 1, 3, 4, 5, 6, 7 with no iron added, is 5.3. Also the chlorosis produced by treatments 1, without micronutrient elements added; 3 with manganese; 4 with boron; 5 with copper; and 6 with Zn; make a total of averages of 31.9. This was antidoted by 5 p.p.m. of iron added in the treatment 8, value 0, in which all the micronutrient elements were added.

Evidence is given to definitely establish that, in the pineapple plant, iron antidotes the action of the chlorosis-producing elements copper, manganese and boron, when these are in a toxic concentration in the nutrient solution.

The treatments of zinc and aluminum showed beneficial effect against chlorosis.   Treatments 6 and 7 gave lower values of chlorosis than treatment 1.
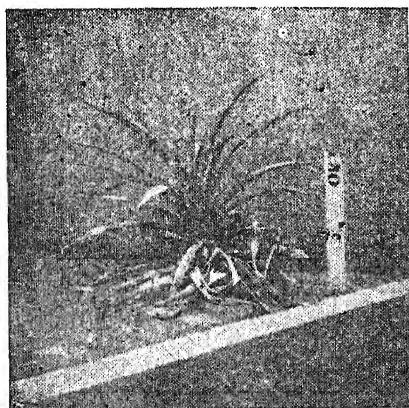
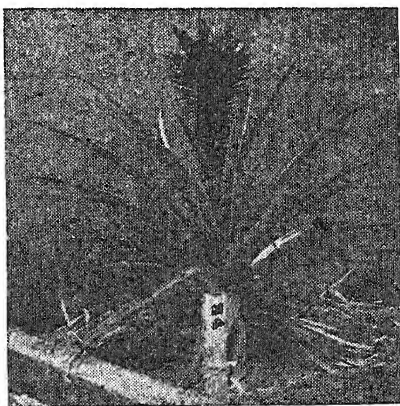### PINEAPPLE PLANTS, 493 DAYS AFTER PLANTING
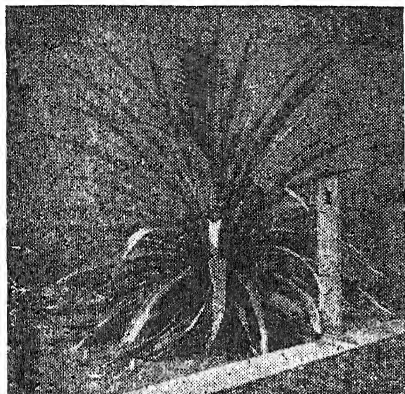
A.   Treatment 1.
No micronutrient elements added.
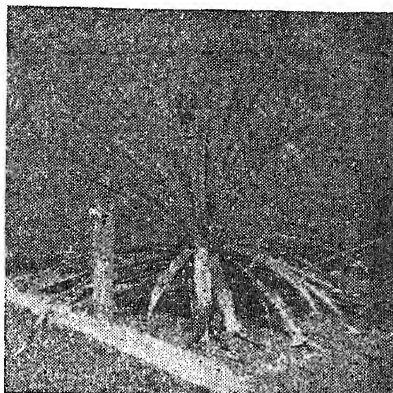
B.   Treatment 3.
2 parts per million manganese added.

C.   Treatment 10.
No iron added.

D.   Treatment 11.
1 ppm of iron added.

FIGURE 5.—Treatments 10 and 11, each contain: 5 ppm of manganese, 2 ppm of copper, ½ ppm of zinc, ½ ppm of boron, ½ ppm of aluminum.

Note that "C" is strongly chlorotic and necrotic, and has not flowered yet; the plant in "D" is heathly, and produced a fruit better than in "A" and "B". The plant, fruit and crown of "A" are better than those in "B".   The presence of iron in "D" counteracted the detrimental action of other micronutrient elements affecting the plants in "A", "B" and "C".

PINEAPPLE PLANTS, 493 DAYS AFTER PLANTING
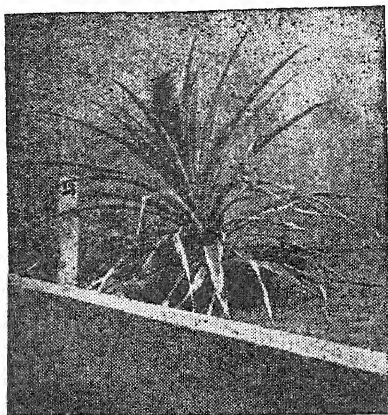
E. Treatment 5.
2 ppm of copper added.
The plant is necrotic. The fruit and crown are very small.
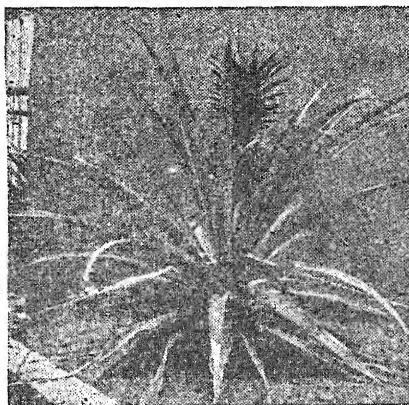
F. Treatment 9.
No copper added.
The plant is healthy. Fruit and crown are normal.

FIGURE 6.—Compare the plant in "E" with the plant in "D", figure 5. The detrimental effect of copper on plant and fruit is noticed.

2. Root Growth

The roots started to come out on the eighth day. On the 79 day they were rather crowded in the quart-jar vessels and were to be changed to gallon jars.

Browning or burning of the root tips was noticed in some treatments. The severity of this injury is expressed numerically in table III. This root-rot injury in pineapple roots was noticed by Schapelle (71) in cultures deprived of copper.

As is shown in table III, up to the 79 day the solution had not been aerated, and on that day the root injury due to this burning or browning of the tips was noticed in the absence of iron and copper.

In nonaerated solutions, copper showed the greatest beneficial action. Treatment 5, in which copper was added, showed almost no injury; while treatment 9, with no copper added, showed a very high severity of injury.

Aluminum showed the highest intensity of injury to roots, in the nonaerated cultures. Zinc also showed injurious effects in nonaerated solutions. Compare the results of treatments 6 and 7 with treatment 1.

The aeration of the solution, as shown on the 108 day, tended to reduce the injury in all treatments. The aluminum treatment recovered rapidly and instead of the detrimental action a highly beneficial action was noticed. In the copper treatment it was the reverse, the beneficial action of copper turned into a detrimental effect. Zinc showed recovery also.

TABLE No. III

OBSERVATIONS ON THE ROOTS

Treatment		Total Browning of Roots		Average volume in ml.	Appearance on the 149 day
Number	Elements	unaerated 79 day	aerated 108 day		
1	ME	6	6	125	Good
2	Fe	1	0	114	Very good
3	Mn	3	3	159	Good
4	B	4	4	137	Short
5	Cu	1	5	86	Hairless, stiff & brown
6	Zn	7	4	85	Good
7	Al	10	2	173	Very good
8	ME	3	3	72	Good
9	-Cu	8	1	167	Very good

IRON MANGANESE SERIES. Mn. 5 p. p. m.

10	Fe 0 p p m	1	0	77	Good
11	Fe 1 p p m	0	0	73	Good
12	Fe 3 p p m	0	0	101	Good
13	Fe 5 p p m	0	0	86	Good
14	Fe 10 p. p. m	0	0	70	Good

Table I gives the concentrations of nutrient elements in each treatment.

Numerical evaluation of browning based on: Very slight = 1, slight = 2, medium = 3.

Volume of roots obtained by displacement of water.

The iron treatments showed beneficial action with aerated as well as non-aerated solutions. Manganese and boron showed some beneficial action.

This interesting response of the micronutrient elements, as related to the aeration of the nutrient solution, points out that as far as root growth is concerned, an oxidation-reduction process plays an important role. It seems that iron and copper have a beneficial oxidizing action under anaerobic conditions; while aluminum and zinc do not show this property. On the other hand, the action of aluminum and zinc has a beneficial effect under the conditions of ample oxygen supply in aerated solutions.

The findings of Loehwing (54), Clark and Shive (19), and Bryant (14), as to the beneficial effect of nonaerated nutrient solution on root growth, should be conditioned by saying that copper or iron are required to be present.

The beneficial action of copper in raw peat soils, pointed out by Allison, Bryan and Hunter (2), may be attributed in part to the anaerobic conditions of these soils. The detrimental effect of aluminum on root growth, as noticed by Blair (10) Barnette (6), Abbot (1), and McGeorge (57), can be explained also as due to conditions of low oxygen supply in the nutrient solution. On the other hand, the beneficial action of aluminum as observed by Schappell (71) is due to the presence of a good supply of air.

The reports of Liebig, Vanselow and Chapman, as quoted by Arnon (3), on copper toxicity in citrus roots, and the stimulating action of aluminum, are confirmed by the results shown in these experiments as to the action of copper and aluminum on root growth under aerobic conditions. That is, aluminum and copper counteract each other under either aerobic or anaerobic conditions.

The volumes of roots, as shown in table III, point out that copper and zinc tend to inhibit root growth; while aluminum, boron and manganese appear to stimulate it. Iron does not show any particular effect, for treatment 2 showed about the same results as treatment 1.

The combined effect of copper and zinc tended to produce low volume of roots in treatments 8 and 10 to 14. Treatment 9 without copper showed the best volume.

3. *Flowering and fruiting*

The first plant to flower and bear fruit was one of culture treatment 14, with 5 p.p.m. of manganese and 10 p.p.m. of iron. The fruiting stalk appeared at the 214 day, and the mature fruit was harvested on the 323 day. Table IV shows the average day when

the flowering started, and the average day when the fully mature fruit was picked from the plant in each treatment. The average weight of the fruit at maturity with and without crown and the weight of the crowns are given for each treatment. The figures on table IV show very interesting results.

TABLE No. IV

FLOWERING AND FRUITING

MATURE FRUITS—AVERAGE FOR THREE PLANTS

Treatment		Days Taken		Weight in Grams		
Number	Elements	To Flower	To give mature fruit	Fruit	Crown	Fruit & Crown
1	ME	424	536	1,033	389	1,422
2	Fe	423	534	1,170	274	1,444
3	Mn	423	539	1,130	263	1,393
4	B	494	611	1,165	275	1,440
5	Cu	519	624	863	258	1,121
6	Zn	450	555	853	246	1,099
7	Al	431	533	1,209	338	1,547
8	ME	405	533	1,067	199	1,266
9	—Cu	431	529	1,353	357	1,710

IRON-MANGANESE SERIES—5 P. M. OF MANGANESE

10	Fe 0 p. p. m	570	675	521	160	681
11	Fe 1 p. p. m	421	523	1,126	258	1,384
12	Fe 3 p. p. m	412	519	1,133	319	1,452
13	Fe 5 p. p. m	412	514	1,156	331	1,487
14	Fe 10 p. p. m	409	509	1,230	376	1,606

See Table I for concentrations of elements in each treatment.

The treatments 11 and 12 showed flowering and fruiting earlier; and the more iron, the earlier.

Copper, boron, and zinc showed a retarding influence on flowering and fruiting, as shown in treatments 4, 5, and 6 as compared with 1. Copper exerted the most detrimental effect. See figures 5 and 6. It is remarkable that no retarding action was shown by manganese in treatment 3, thus showing that it is the retarding effect of copper, boron and zinc that have been antidoted by iron in treatments 8, 9, 11, 12, 13, and 14. The retarding action in treatment 10 may be attributed to the indirect effect of manganese–chlorosis and the retarding effect of other micronutrient elements added.

The treatments with iron showed the earlier flowering and fruiting, but it appears that iron and manganese have an additive action superior to their independent action. Compare treatments 2 and 3

where these elements are alone, one in each treatment, with 8 and 11 to 14, where they are together, and against the retarding influence of copper, boron and zinc.

The weights of fruits, either with or without crown, showed to be affected quite similarly to flowering and fruiting. The treatments of copper and zinc gave the lowest yield comparing treatments 5 and 6 with 1.

It should be pointed out that the minus-copper treatment 9 showed the highest yield. The aluminum treatment also gave a high yield. Iron and boron favored high yields also.

The production of fruits was not so badly affected by the injury on roots and by chlorosis when the injury did not reach the advanced stage of destroying the plant to a certain degree, as it did in the plus-copper treatment 5 and in the no-iron treatment 10. In the iron-manganese series the weight of crop increased with the increase in iron.

The desirable vigorous crown was at its best in the manganese and iron treatments, where increasing quantities of iron were added. The treatments without zinc did not show detrimental action on the crown. It was expected that treatments without zinc might give short crowns, Hopkins et al (40), connecting it with the "little leaf" disease in citrus, or any distortion in the leaves as discussed by Hoagland (34), due to lack of zinc in the nutrient solution.

It is noticed that the aluminum treatment produced very good fruits as to weight of the fruit and of the crown. This may be due to its beneficial effect on roots when aerated.

4. *Composition of the juice of the ripe fruits.*

Table V shows the degree brix of the juice, a figure approaching the per cent of total solids by weight in solution, called also "apparent gravity solids" in sugar technology. The total sugars in juice are expressed as invert sugar. The ratio of total sugar to brix has been calculated and multiplied by 100, so as to show the relation between sugars on the total (gravity) solids, and so as to give a figure independent of dilution. This may be called the "gravity purity of invert sugars". It is the figure that should give the best criteria of the quality of the juice as to its sugar content.

The percentage acidity is reported on the basis of citric acid as discussed in the Experimental Section. The percentage of reducing sugar in the juice is also given.

TABLE No. V

COMPOSITION OF THE JUICE OF THE RIPE FRUITS

Treatment		Juice Analysis—Average for three Plants				
Number	Elements	Reducing Sugar %	Total Sugar as Invert Sugar %	Degree Brix	o/o of solids which are Sugars	Acidity as Citric Acid %
1	-ME	2.75	10.70	15.7	68.2	0.73
2	Fe	2.61	9.80	13.1	74.8	0.67
3	Mn	3.02	9.34	14.7	63.5	0.76
4	B	3.29	10.84	14.6	74.0	0.68
5	Cu	2.46	9.06	12.7	71.3	0.66
6	Zn	1.95	7.60	11.5	66.0	0.88
7	Al	2.45	8.28	11.7	70.8	0.74
8	ME	3.73	11.48	14.8	77.5	0.61
9	-Cu	2.72	9.80	13.3	73.7	0.68

IRON-MANGANESE SERIES—5 P. P. M. OF MANGANESE

10	Fe 0 p. p. m.	2.47	9.23	13.9	66.4	0.72
11	Fe 1 p. p. m.	4.38	9.28	12.6	73.7	0.61
12	Fe 3 p. p. m.	3.05	11.22	15.6	78.8	0.63
13	Fe 5 p. p. m.	2.66	11.35	14.8	76.8	0.64
14	Fe 10 p. p. m.	3.22	11.50	14.3	80.4	0.53

See Table I for concentrations of elements in each treatment.

The best quality of the fruits is associated with the highest ratio of sugar content to total dissolved solids, and the lowest acidity in the juice.

The sugar content of the juice was lowest in the zinc, manganese and aluminum treatments. The treatment with no micronutrient elements added showed a low sugar content. The acidity was higher in these treatments. The copper treatment showed also some detrimental action, as shown by comparing treatment 5 with copper, and treatment 9 without copper. Iron and boron showed beneficial action for increasing the sugar content and lowering the acidity. Compare treatments 2 and 4 with 1.

The iron-manganese series showed low quality of juice in treatment 10, without iron. The other treatments, 11 to 14, showed increasing content of sugar and decreasing content of acid, with the increase in iron. Iron was the most important agent of high quality. All treatments with iron were superior to treatments without iron.

The reserve micronutrient elements in the slip were not sufficient to produce good quality. The values for treatment 1 showed rather low sugar content and a high acidity. Compare treatment 1 with treatment 8, which gave the best analysis next to number 14, that had 10 p.p.m. of iron. It seems that the chlorosis-producing effect of the elements is related to low sugar content, and high acidity.

V — SUMMARY AND CONCLUSIONS

A. *Work done*

Pineapple plants were grown in nutrient solutions from uniform and healthy slips used as the propagating organ. The solutions were prepared with a mixture of macronutrient elements containing ammonia and nitrate nitrogen, potassium, phosphorus, magnesium, calcium, and sulfur. The solutions proved to be good for the growth of pineapples.

Fourteen different treatments of the micronutrient elements: iron, manganese, boron, zinc, copper and aluminum, were used in triplicate. Combinations of these elements were made in order to trace their effect, either toxic or beneficial, on pineapple plant growth and production, on root growth, on flowering and fruiting, and on the quality of the fruit. The antidoting effect of iron against the chlorosis-producing action of manganese was also studied. Plants also were grown without adding micronutrient elements to the nutrient solution.

B. *Conclusions* (See footnote on page 2̶3̶7̶)[2]

1. Iron antidotes the chlorosis-producing action of manganese in pineapple plants. With 5 parts per million of soluble manganese in a nutrient solution containing one-half part per million of boron, of zinc, and of aluminum, and 2 parts per million of copper, severe chlorosis and necrosis appeared before the pineapple plant was able to attain full growth. But, with one part per million of soluble iron humate added to a similar treatment, a healthy, normal plant was produced.

2. Iron counteracts the chlorosis-producing effect manifested by copper and boron. It raises their chlorosis-producing level.

3. Copper and manganese, at a concentration of 2 parts per million in the nutrient solution, produce strong chlorosis if iron is not present.

4. Aluminum and zinc show beneficial effects against chlorosis.

5. Iron shows no toxic effects on pineapple plants when added as the only micronutrient element to the culture solution, up to 5 parts per million. If chlorosis-producing elements like manganese or copper are present, higher concentrations are beneficial.

6. The reserve iron content in slips of the variety "Smooth Cayenne" is quite enough to counterbalance the detrimental chlorosis-producing effect of the other reserve micronutrient elements in the slip. However, plants grown in nutrient solutions deprived of all micronutrient elements produced fruits of lower sugar content than those supplied with micronutrient elements.

7. Pineapple plants respond to increase in iron in the nutrient solution, giving increase in green color, in yield, in sugar content and in decreased acid content. Treatments with a constant supply of manganese and other micronutrient elements, as explained above in (A), and with iron added in concentrations ranging from 1 to 10 parts per million, showed that the above-mentioned beneficial effect varied directly with the increase in iron added to the nutrient solution.

8. Iron prevents browning of root tips, or root rot, of pineapple plants under either anaerobic or aerobic conditions. Manganese and boron show also a somewhat beneficial action.

9. Copper has a highly beneficial action in preventing root-rot injury, when under anaerobic conditions. However, when the nutrient solution is well aerated the effect of copper is somewhat detrimental to the roots. Nonaerated solutions must be well supplied with copper and iron.

10. Aluminum has a beneficial action on root health and volume under the aerobic conditions of well aerated nutrient solutions, but it is highly toxic to the roots under anaerobic conditions.

11. Zinc exerts a somewhat detrimental action on roots under anaerobic conditions of the nutrient solution.

12. It seems that as far as root growth is concerned aeration plays an important role; copper having a beneficial action under anaerobic conditions; and aluminum when the nutrient solution is well aerated. Iron, manganese and boron are beneficial under any of the two conditions of oxygen supply.

13. Aluminum and manganese promote increased volume of roots when the nutrient solution is well aerated. Copper and zinc tend to reduce the volume of roots, but iron and boron show no direct effect.

14. Iron has a beneficial effect on early flowering and early maturity of the pineapple fruit.

15. Copper, zinc and boron, and the chlorosis-producing effect of manganese, have a retarding effect on flowering and fruiting. In

this action they are counteracted by iron. Aluminum shows no specific effect upon flowering and fruiting.

16. Zinc and copper tend to produce low yields. Aluminum and boron increase the yield. Manganese does not show specific effect on yield when added alone to the nutrient solution.

17. Zinc, copper, manganese and aluminum show a tendency to produce fruits with low sugar content and high acidity.

18. Iron and boron show a beneficial effect by increasing the sugar content and lowering the acidity. Iron is an important agent of high quality of pineapple fruits.

19. Zinc deficiency in the nutrient solution shows no signs of anatomical abnormalities in the pineapple plant.

20. Chlorosis in pineapple plants causes lower sugar content and higher ratios of acid to sugar in the fruit.

21. Experimentation on the mutual effect of the micronutrient elements on pineapple should take into consideration the reserve of elements in the planted slips.

22. The antidoting action of iron against the detrimental effects of copper should be studied by using varying concentrations of iron and different concentrations of copper.

23. Field experiments with iron humate added to the pineapple soils should be made to study its effect under soil conditions.

24. More available iron in soils will greatly improve the Puerto Rican pineapple crops.

NOTE: The above-mentioned conclusions on the independent action of the micronutrient elements refer to treatments in which they were added separately as the only micronutrient elements to the nutrient solutions in which the pineapple plants were grown. The concentrations used for each element were: 5 ppm Fe, 2 ppm Mn, 1 ppm B, 2 ppm Cu, 1 ppm Zn, and 1 ppm Al.

C. SYNOPSIS OF THE EFFECT PRODUCED BY MICRONUTRIENT ELEMENTS ON PINEAPPLE PLANTS, WHEN THE ELEMENTS ARE ADDED INDEPENDENTLY TO THE NUTRIENT SOLUTIONS

Element	PPM added to the nutrient solution	Chlorosis	Root-rot injury Solutions non-aerated	Root-rot injury Solutions aerated	Volume of roots aerated solutions	Early flowering and fruiting	Production Yields	Production Quality
Iron	5	Prevented Chlorosis	Prevented injury		No effect	Beneficial effect	High	High
Manganese	2	Severe Chlorosis	Beneficial effect against injury		Good	Retarding due to chlorosis	Not Specific	Low
Boron	1	Produced Chlorosis	Beneficial effect against injury		Not effect	Retarding effect	High	Good
Copper	2	Severe Chlorosis	Beneficial effect against injury	Detrimental effect	Detrimental effect	Retarding effect	Low	Low
Zinc	2	Prevented Chlorosis	Detrimental effect	No detrimental effect	Detrimental effect	Retarding effect	Low	Low
Aluminium	1	Prevented Chlorosis	Detrimental effect	Beneficial against injury	Beneficial effect	No effect	High	Low

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CALCIUM-BORON RELATIONSHIPS IN THE NUTRITION OF CORN AND
THE DISTRIBUTION OF THESE ELEMENTS IN THE PLANTS

by Ernesto Hernández-Medina and John W. Shive

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CALCIUM-BORON RELATIONSHIPS IN THE NUTRITION OF CORN AND THE DISTRIBUTION OF THESE ELEMENTS IN THE PLANT [1]

ERNESTO HERNÁNDEZ-MEDINA [2] AND JOHN W. SHIVE [3]

The literature dealing with the inorganic nutrition of plants reveals that great attention has been given to the important role that the so-called trace elements play in the metabolic activities of different species of plants. Boron is one of these elements that has received particular attention in this regard. Both qualitative evidence and quantitative evidence (23, 28, 32, 33, 42, 43, 50) show that this element is indispensable for normal growth and development of plants and, therefore, it may be added to the previous list of essential elements (38).

Soils naturally low or deficient in boron have been reported (35, 48) in over half of the 48 states, with large areas occurring along the Atlantic coastal plain, in the Great Lakes region, and in the Pacific Northwest. If boron is not applied in usual fertilizer practices to such deficient or sub-deficient soils metabolic disturbances of the plants are likely to result in abnormal plant development as evidenced by the appearance of boron deficiency symptons and reduced crop production. Naftel (25,26,27) was one of several investigators (5,20,51) to show that liming of soils naturally low or deficient in boron is likely to cause an initiation or accentuation of boron deficiency symptoms in plants growing thereon. He found that in soils low or deficient in boron, liming decreased the water-soluble boron of the soil. On the other hand, White-Stevens (49) found that the effective level of boron in the fertilizer mixture for controlling boron deficiency in deficient soils depended upon the kind of crop grown. This suggests that care should be exercised in the application of fertilizer in soils lacking or low in boron.

The fact that it is virtually impossible (36,38) to distinguish externally between boron and calcium deficiency symptoms has sug-

gested a possible relation between these two elements in plant nutrition. Among the first investigators to suggest a possible association between boron and calcium in the plant were Brenchley and Warington (3,41) using the broad bean as an indicator plant. They suggested that boron either enables the plant to absorb more calcium in a given period of time or permits it to be used more efficiently once it is absorbed by the plant. In the absence of boron, calcium in the roots was not utilized effectively. Later work by Marsh and Shive with corn (17) corroborated the findings of Brenchley and Warington. They found that the presence of an appropriate amount of available boron in the plant tended to maintain, in an available condition, the calcium absorbed even if no additional calcium was supplied to the plant. Evidence is also presented which demonstrates that the boron content of the culture solution did not greatly influence rates of calcium absorption by the plants. In an annual report of the New Jersey Agricultural Experiment Station the suggestion is made that boron may influence the normal metabolism of calcium in plants (29). The suggestion is sustained by the fact that no calcium was found or detected in the cell sap in the meristems of boron deficient cotton plants. Results obtained by Lowenhaupt, working with sunflower (16), with reference to calcium content of tissues are in agreement with the view that there is a relationship between the boron available to the plant and the utilization of calcium. Haas (11) working with citrus and walnuts found no evidence in the leaves of any relationship between the soluble boron and the soluble calcium in the same tissues; while Muhr (24) working with sugar beets, radishes, chickory, turnip, barley, wheat, dandelions, mangels, rutabaga and corn, and Hill and Grant (12) with turnips found that tissue of plants inadequately supplied with boron contained, in general, a higher percentage of calcium than the tissue grown with sufficient boron. Still, there is a diversity of opinion among investigators as to the determinative role of boron in the accumulation of calcium in the plant. Haas (10) working with citrus found that the percentage of total calcium that was water soluble was higher in leaves of plants which received large quantities of boron in the substrate than in the leaves of plants receiving lower levels of boron in the nutrient medium. On the other hand, Holley and Dulin (13), Morris (22), and Dmitriev (7) furnished evidence to demonstrate that the presence or absence of boron in the nutrient media had no influence on the quantity of calcium in the plant tissue analyzed. As has been indicated (31) some disagreement may be due to the use of different species of plants, dif-

ferent growing media (sand and water cultures, in some cases, soil, in others) varying climatic conditions, and differences in the boron concentrations involved.

Later work (4,6,14) has furnished evidence that a definite balance exists between the calcium and boron content of healthy tomato, tobacco and oat plants. Brennan (4) has indicated that normal tomato plants have an intermediate numerical ratio of total calcium to total boron whereas boron deficient plants and boron toxic plants have abnormally high and abnormally low ratios, respectively.

The purpose of this investigation was to study further the calcium-boron relationship in the corn plant and to determine the distribution of these elements in this monocotyledon.

SERIES I

METHODS

Cultural methods—This experiment was the first of two series and was started on October 16, 1945. The plants were grown in sand culture. Corn seeds of Rutgers Hybrid No. 2 were used. They were selected for uniformity in shape and size and were sowed directly in purified sand in 9 inch highly glazed pots. The sand was previously washed with tap water, then treated with 2 per cent sodium hydroxide and left overnight. Following this, it was flushed with distilled water, treated with 2 per cent hydrochloric acid and again left overnight, after which it was completely flushed with distilled water until free from chloride ions as determined by the silver nitrate test. This sand treatment was necessary to remove any materials which might furnish boron and also to free the sand from other nutrients. Four seeds were planted in each pot and the sand was flushed with a dilute nutrient solution of the following composition: KH_2PO_4, 0.0016 M.; $NaNO_3$, 0.0038 M.; $MgSO_4$, 0.0028 M.; $CaCl_2$, 0.0025 M. with the usual traces of iron, zinc and manganese but with no boron. Four days after germination the best three seedlings were left for treatment. The rejected seedling was removed by first flushing the sand with distilled water and then gently pulling it out with its entire root system. The cultures were maintained on the diluted culture solution for a week to develop uniform plants before nutrient treatments were started. At the end of this period all plants appeared vigorous and fairly uniform and the calcium-boron nutrient treatments were begun and maintained for 64 days, using the continuous solution renewal method of Shive and Stahl (39,40). Approximately two liters of nutrient solution per day were used for each culture containing three plants.

In this experiment there was a total of forty-eight cultures, which were divided into six groups of eight cultures each. Each group was grown at a different level of calcium, as follows: 5.0, 10.0, 50.0, 100.0, 250.0, and 500.0 p.p.m. Each of the eight cultures in a given calcium level received boron in eight different concentrations. The levels of boron used were as follows: 0.0, 0.001, 0.01, 0.10, 0.25, 5.0, 10.0, and 20.0 p.p.m. Figure 1 ilustrates the plan of the experiment the number on each block representing culture numbers. The composition of the culture solutions with the different levels of calcium used with each of the eight boron levels is presented in table 1. Salts of analytical grade were used without further repurification. Boron was supplied in the form of boric acid. The ammonium sulfate was used to aid in the prevention of iron-deficiency chlorosis.

At the end of the experimental period, on December 28, when definite symptoms of boron deficiency and toxicity occurred on plants receiving deficient and very high nutrient levels of boron respectively, the plants on the different treatments were harvested. After green and dry weights of the tops had been obtained, the plants were cut with a stainless steel knife into small pieces which were thoroughly mixed so that representative samples could be obtained for analysis.
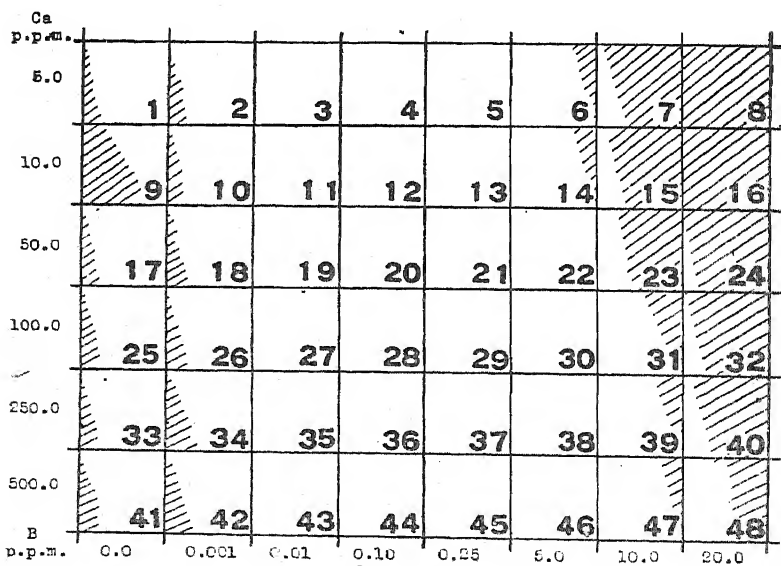

FIG. 1.—Diagram showing calcium and boron treatments and general experimental plan. Relative proportion of shaded area of each block at the left denotes relative degree of boron deficiency symptoms, those at the right boron toxicity symptoms.

TABLE No. 1

COMPOSITION OF CULTURE SOLUTIONS AT EACH OF THE 6 DIFFERENT NUTRIENT LEVELS OF CALCIUM USED WITH EACH OF 8 DIFFERENT BORON CONCENTRATIONS OF 0.0, 0.00¹, 0.01, 0.10, 0.25, 5.0, 10.0, AND 20.0 P. P. M.*

Culture Numbers	Calcium Levels	Molar Concentration of Salts						
		KH_2PO_4	K_2SO_4	$NaNO_3$	$MgSO_4$	$CaCl_2$	$CaSO_4$	$(NH_4)^2 SO_4$
	p. p. m.							
1–8	5	.00113	.00113	.0085	.00113	.000125	....	.0004
9–16	10	.00113	.00113	.0085	.00113	.00025	....	.0004
17–24	50	.00113	.00113	.0085	.00113	.00125	....	.0004
25–32	100	.00113	.00113	.0085	.00113	.0025	....	.0004
33–40	250	.00113	.00113	.0085	.00113	.00625	....	.0004
41–48	500	.00113	.00113	.0085	.00113	.00625	.00625	.0004

* TRACE ELEMENTS:– Each nutrient solution contained 1 p. p. m. iron, 0.5 p .p. m. zinc, and 0.25 p. p. m. manganese used in the form of sulfates.

In order to determine the soluble boron and calcium in the plant tissue, a 50–gram sample of fresh tissue from each culture was frozen in a refrigerator immediately at time of harvest and kept at approximately minus 18°C until ready for analysis. In addition, 100–gram samples of fresh tissue were weighed and dried as described below to be used in the determinations for total boron and total calcium in each aliquot.

At the time of harvest one of the three plants from a culture receiving a high and a low boron level in each calcium series was harvested separately and fractionated into top leaves, middle stem and lower stem in order to make a preliminary study of the distribution of the total boron and calcium in these portions of the plant. The fraction designated as "top leaves" consisted of the leaves extending above and including the apical meristem; the "middle stem" fraction consisted of the culm tissue including leaf sheath but without blades extending from the base of the apical meristem to the node second removed from the sand surface; the "lower stem" fraction consisted of the lower two nodes and internodes of the culm including leaf sheaths but without blade tissue.

Chemical Methods—Dry weights of the 100–gram samples of green tissue taken for analysis at the time of harvest were obtained after oven drying at 70°C for 48 hours. The dried tissue was then ground in a semi-micro Wiley mill to pass a 40 mesh screen, after which 2–gram samples were weighed into porcelain crucibles and ignited in a muffle furnace starting at room temperature and gradually raising the temperature to 600°C. The samples were held at this temperature for approximately 6 hours which resulted in complete ashing. The ash was dissolved in 20 ml. of (1 +4) HCl, transferred

to a 100 ml. volumetric flask and made to volume. Aliquots of this solution were then taken for determination of total boron and total calcium.

For the determination of tissue contents of soluble boron and soluble calcium, the frozen 50–gram tissue samples were thawed and then placed in a piece of muslin of suitable size fitted in a nickel plated steel cylinder 5.71 centimeters inside diameter, covered with 40 ml. of distilled water, and subjected to a pressure of 2500 pounds per square inch for one minute in a Carver Press. The press cake was washed twice more with 40 ml. of distilled water and subjected to the same pressure but after the last washing the pressure was maintained for two minutes. The extracted plant juices and washings were passed through a quantitative Whatman number 5 filter paper using suction. The press cake was then removed from the muslin and together with the filter paper was oven-dried at 70°C for 48 hours. After drying, press cake and filter paper were ignited in a muffle furnace and the ash was dissolved and the solution made to volume as previously described. Aliquots of these samples were taken for the determinations of boron and calcium, respectively. Soluble boron and soluble calcium contents of the tissue were determined by the differences between the analytical values of these two elements in the unextracted sample and the extracted press cake.

In this work the soluble portions of boron and of calcium are considered to be those which were extracted from the tissue samples under pressure by the method described and these soluble portions are also considered to be the active portions in the tissue at least at the time of harvest. Those portions of the calcium and boron which remain in the plant tissue in the press cake after extraction of the juices by the above method are considered to be the insoluble and inactive portions.

The official micro-method of the Association of Official Agricultural Chemists (1) with a few minor changes was used for the calcium determinations. Since it is not necessary to remove the silicon dioxide, this step was omitted (21). Coprecipitation of the silicon dioxide with calcium oxalate does not interfere in the titration of the oxalate ions by the permanganate ions. To obtain greater accuracy in the results 0.01 N $KMnO_4$ was used for titrating the oxalate ion instead of 0.02 N solution recommended in the official method.

The Berger and Truog (2) colorimetric method was used for the quantitative determination of boron with modifications similar to those of Marsh and Shive (17) except that 10 ml. quantities of sul-

furic acid-quinalizarin reagent were used with 1 ml. of the test solutions instead of the 50 ml. quantities used by Marsh and Shive. The 50 ml Nessler tubes used for color comparison of standards and test solutions were kept tightly closed during the determinations. Sulfuric acid, 95.5 per cent by weight, was used in this method instead of the 98 per cent acid employed by Berger and Truog.

RESULTS

Character of Plants after Treatment.—The relative intensities of the visible symptoms of metabolic disorder resulting respectively from deficient and excess nutrient concentrations of boron in the culture solution are represented by the shaded areas in figure 1. All the plants at the two lowest boron levels, 0.00 and 0.001 p.p.m. exhibited boron deficiency symptoms. However, as is indicated in figure 1, none of these boron deficient plants were very seriously injured and, moreover, no definite relation is observable between severity of boron deficient symptoms and relative calcium concentrations of the substrate. The injury due to boron deficiency which occurred in boron deficient cultures in this experiment ranged from slight to medium intensity and was not so severe as that in similar nutrient treatments in the experiment to be reported later in this paper. The boron deficiency symptoms were characterized by the appearance of elongated white transparent interveinal stripes on the newly formed leaves (44) and were similar but not quite so severe as those on leaves of plants shown in figure 2, which were grown in a subsequent experiment. The roots of the boron deficient plants were slightly brown, and the root systems were not so extensive as those of plants receiving boron. These symptoms were especially severe at the low calcium levels probably as a result of both boron and calcium deficiencies.

It has been reported (46) that under the short day conditions of spring and autumn the onset of boron deficiency symptoms is delayed as compared with long day conditions of summer. Since this experiment was run during the fall season it is possible that the short length of day may account for the fact that the boron deficiency symptoms were not so severe as those which developed in boron deficient cultures grown in the subsequent experiment conducted under the long day conditions of late spring. No distortion or injury was observed in apical meristems of boron deficient plants.

Figure 1 shows that slight boron toxicity symptoms appeared in cultures number 6 and number 14 receiving 5.0 p.p.m. boron at the two lowest calcium levels. Plants in other cultures grown at this boron level exhibited no toxicity symptoms. At the boron concentrations

of 10.0 p.p.m., boron toxicity symptoms were more severe than at 5.0 p.p.m. and appeared to some extent in plants at all calcium levels. At the boron level of 20.0 p.p.m. the metabolic disturbances due to the high boron concentration were most pronounced and were especially severe at the three lowest calcium levels.

The plants grown at 20.0 p.p.m. boron and with 5.0 p.p.m. of calcium (figure 3) showed more serious symptoms of boron toxicity than the plants grown at the same boron level but with 500.0 p.p.m. calcium (figure 4). Boron toxicity symptoms consisted of stunted

FIG. 2.—Typical boron deficiency symptoms of growing point and top leaves of corn plant grown at nutrient levels of 250.0 p.p.m. calcium and 0.001 p.p.m. boron.

growth, pale yellow-green color of the youngest leaves with yellow colored, dying and dead marginal tissue appearing largely on the older leaves. Typical boron toxicity symptoms are shown in figure 5 on the leaves of plants grown in a subsequent experiment. The root system of boron toxic plants were relatively small and the roots were brown in color. The appearance of these root systems was similar to those of plants receiving similar treatments and grown in a subsequent experiment.

Calcium deficiency symptoms were observed to some extent in the younger leaves of plants receiving 5.0 and 10.0 p.p.m. calcium. These symptoms consisted of distortions, breaks, and colorless areas

FIG. 3.—Corn plants grown in culture number 8 at nutrient levels of 5.0 p.p.m. calcium and 20.0 p.p.m. boron.

of tissue on the margins and tips of the leaves. These calcium deficiency symptoms are similar to, but not so serious as those which occurred on leaves of plants grown in a subsequent experiment. (Figure 6).

With regard to green and dry weights of the whole tops of the plants grown with the various calcium-boron nutrient levels it was found that with few exceptions green and dry weights increased as the calcium concentration of the substrate increased at any given boron level. The green and dry weights of the plants receiving the highest nutrient levels of boron 5.0, 10.0 and 20.0 p.p.m. within
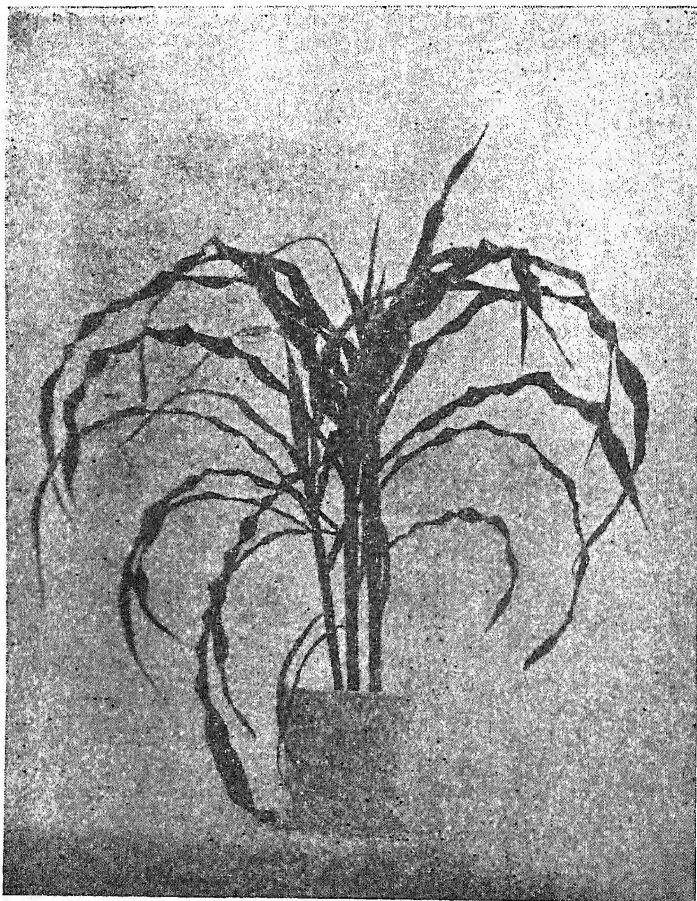
FIG. 4.—Corn plants grown in culture number 48 at nutrient levels of 500.0 p.p.m. calcium and 20.0 p.p.m. boron showing mild symptoms of boron toxicity.

a given calcium level were considerably lower than the weights of those plants receiving lower nutrient levels of boron.

Results of Chemical Analyses.—The results of quantitative analyses for boron and calcium contents of the tissues, both total and soluble, are presented in table 2. It appears from the analytical data that both total boron and soluble boron at any given nutrient level of boron are more or less independent of the calcium concentration in
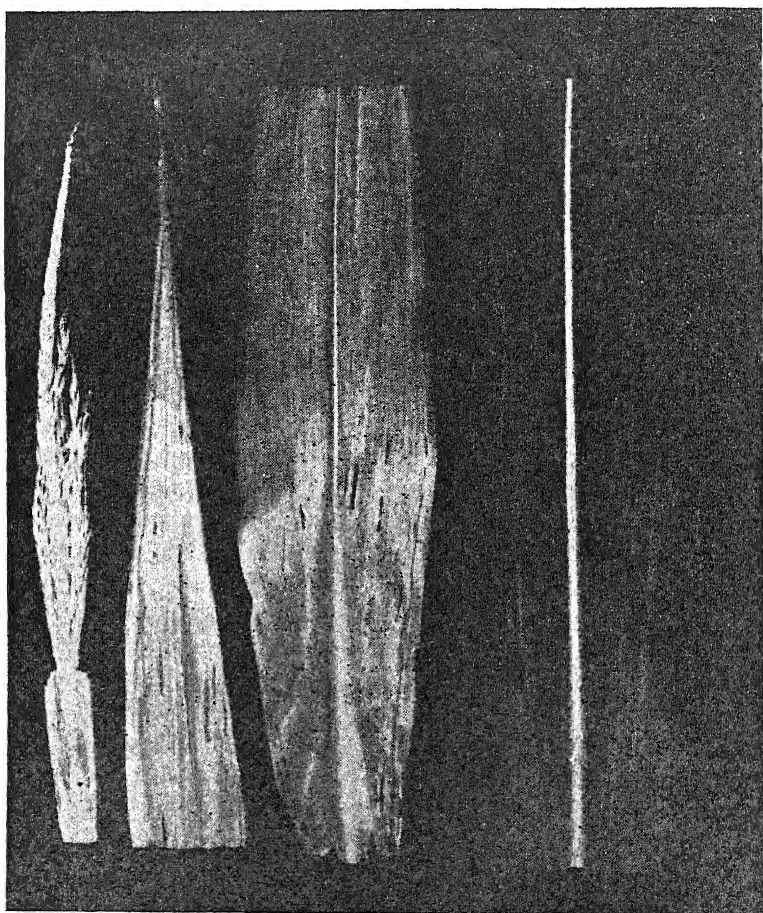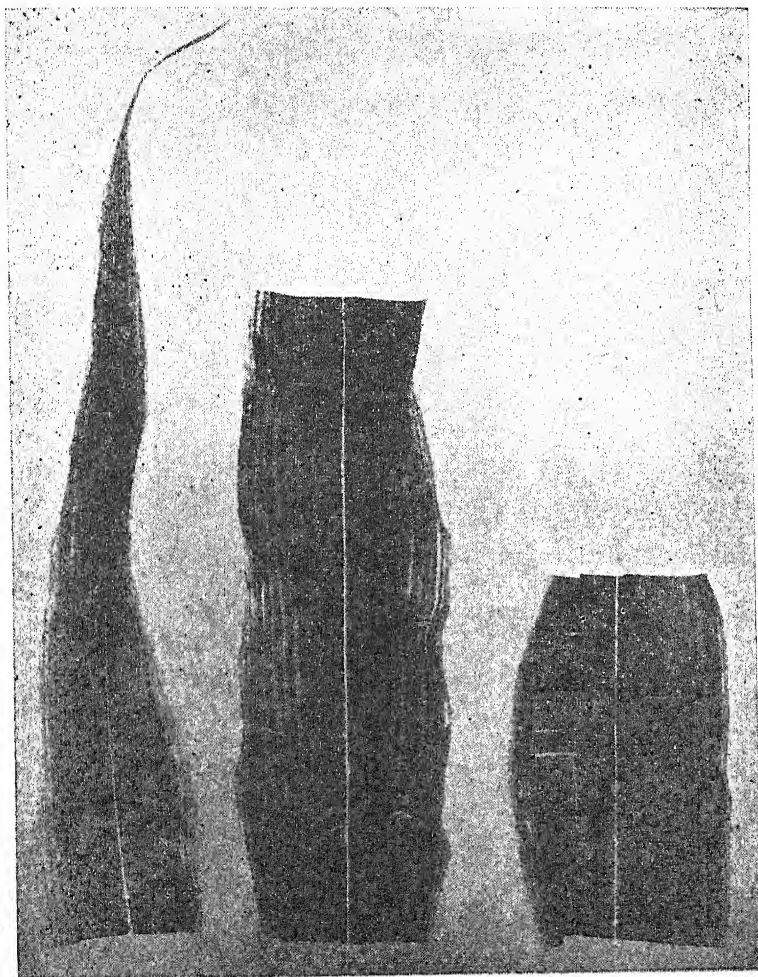

FIG. 5.—Typical boron toxicity symptoms of lower leaves of corn plant grown at nutrient levels of 100.0 p.p.m. calcium and 20.0 p.p.m. boron.

the substrate.   However, the plants receiving highest nutrient con-
centrations of boron (20.0 p.p.m.) contained somewhat lower tissue
contents of boron, both total and soluble, when grown at the highest
calcium level (500.0 p.p.m.) than plants grown at equivalent boron
level but at lower calcium concentrations.   This suggests that boron
accumulation by the plant was modified in some way at the highest
calcium level.   These results support the qualitative observations
wherein boron toxicity symptoms of plants receiving the highest

FIG. 6.—Leaves showing well defined calcium deficiency symptoms
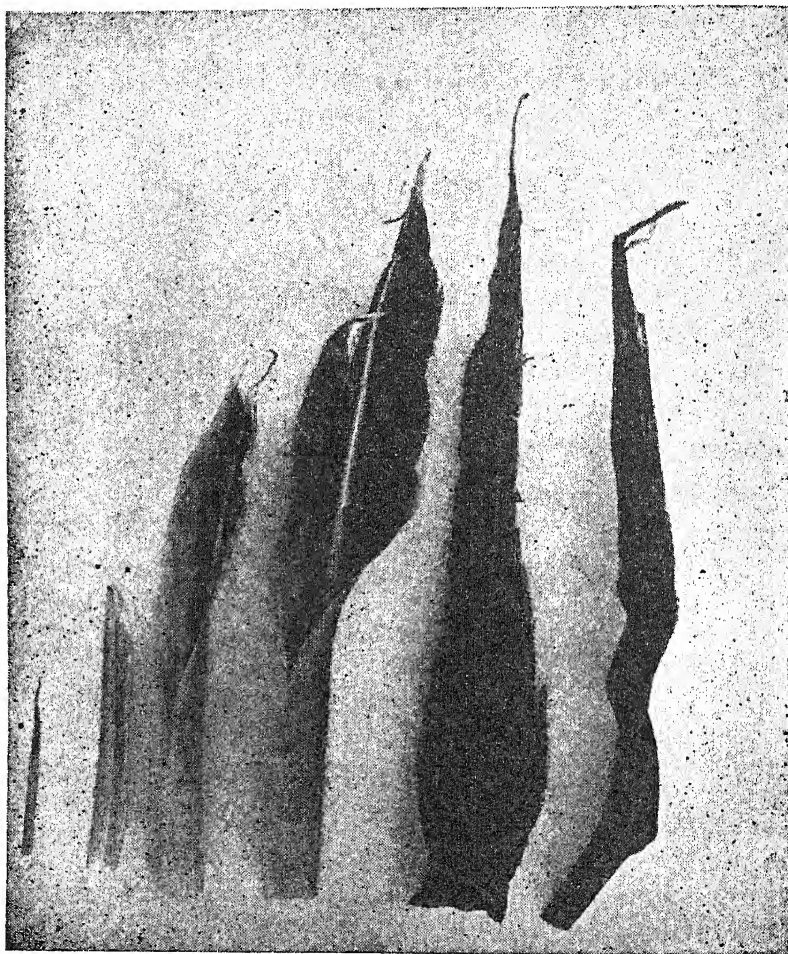of corn plant grown at nutrient levels of 5.0 p.p.m. calcium
and 0.25 p.p.m. boron.

TABLE No. 2

TOTAL AND SOLUBLE CALCIUM AND BORON IN MILLIGRAMS PER GRAM
DRY TISSUE OF WHOLE TOPS OF CORN PLANTS OF SERIES I

Treatment		Total	Soluble	Soluble B	Total	Soluble
Ca	B	B	B	Per cent of Total	Ca	Ca
p. p. m.	p. p. m.	mgm. p. g.	mgm. p. g.	Per cent	mgm. p. g.	mgm. p. g.
5	0.0	0.010	0.002	20.0	0.84	0.53
5	0.001	0.015	0.007	46.6	1.08	0.56
5	0.01	0.020	0.008	40.0	0.95	0.63
5	0.10	0.023	0.012	52.1	1.14	0.77
5	0.25	0.030	0.016	53.3	1.36	1.00
5	5.0	0.300	0.206	68.8	1.00	0.51
5	10.0	0.450	0.388	86.2	1.18	0.85
5	20.0	0.750	0.567	74.6	0.91	0.54
10	0.0	0.010	0.002	20.0	1.63	1.10
10	0.001	0.015	0.006	40.0	1.41	0.84
10	0.01	0.020	0.009	45.0	1.25	0.71
10	0.10	0.023	0.015	65.2	1.51	1.06
10	0.25	0.030	0.016	53.3	1.94	1.24
10	5.0	0.174	0.059	33.9	1.57	1.17
10	10.0	0.424	0.266	62.7	1.65	1.13
10	20.0	0.997	0.610	61.1	1.58	1.02
50	0.0	0.020	0.008	40.0	3.69	2.12
50	0.001	0.020	0.009	45.0	3.11	1.97
50	0.01	0.025	0.013	52.0	3.10	2.02
50	0.10	0.030	0.017	56.6	4.00	2.30
50	0.25	0.031	0.019	61.2	3.09	1.27
50	5.0	0.175	0.119	68.0	3.53	2.61
50	10.0	0.424	0.358	84.4	3.75	2.09
50	20.0	0.765	0.557	72.2	4.59	2.76
100	0.0	0.015	0.008	53.3	5.18	3.22
100	0.001	0.015	0.009	60.0	4.93	2.34
100	0.01	0.025	0.015	60.0	4.75	2.75
100	0.10	0.032	0.019	59.3	4.78	2.88
100	0.25	0.040	0.024	60.0	4.72	3.93
100	5.0	0.250	0.204	80.0	5.62	3.75
100	10.0	0.449	0.384	85.5	4.65	3.25
100	20.0	0.750	0.574	76.5	6.72	4.25
250	0.0	0.015	0.008	53.3	5.80	4.11
250	0.001	0.015	0.007	46.6	6.78	5.40
250	0.01	0.025	0.015	60.0	6.08	4.50
250	0.10	0.035	0.021	60.0	5.93	4.47
250	0.25	0.035	0.018	51.6	5.73	4.13
250	5.0	0.137	0.087	63.5	5.93	4.46
250	10.0	0.350	0.289	82.5	5.73	4.34
250	20.0	0.750	0.531	70.8	8.03	6.01
500	0.0	0.015	0.007	46.6	6.64	5.14
500	0.001	0.015	0.007	46.6	6.70	5.50
500	0.01	0.020	0.012	66.0	6.61	4.95
500	0.10	0.030	0.015	50.0	7.37	5.77
500	0.25	0.030	0.021	70.0	6.33	5.07
500	5.0	0.128	0.093	72.6	7.12	5.74
500	10.0	0.424	0.241	56.8	6.50	5.07
500	20.0	0.624	0.428	68.6	7.93	6.26

nutrient level of boron (20.0 p.p.m.) were less severe at the highest calcium level (500.0 p.p.m.) than at lower calcium levels. These differences in severity of boron toxicity symptoms were also reflected in the green and dry weight yields of the plants. Parks et al working with tomatoes obtained reduced green and dry weights of plants receiving toxic quantities of boron (31).
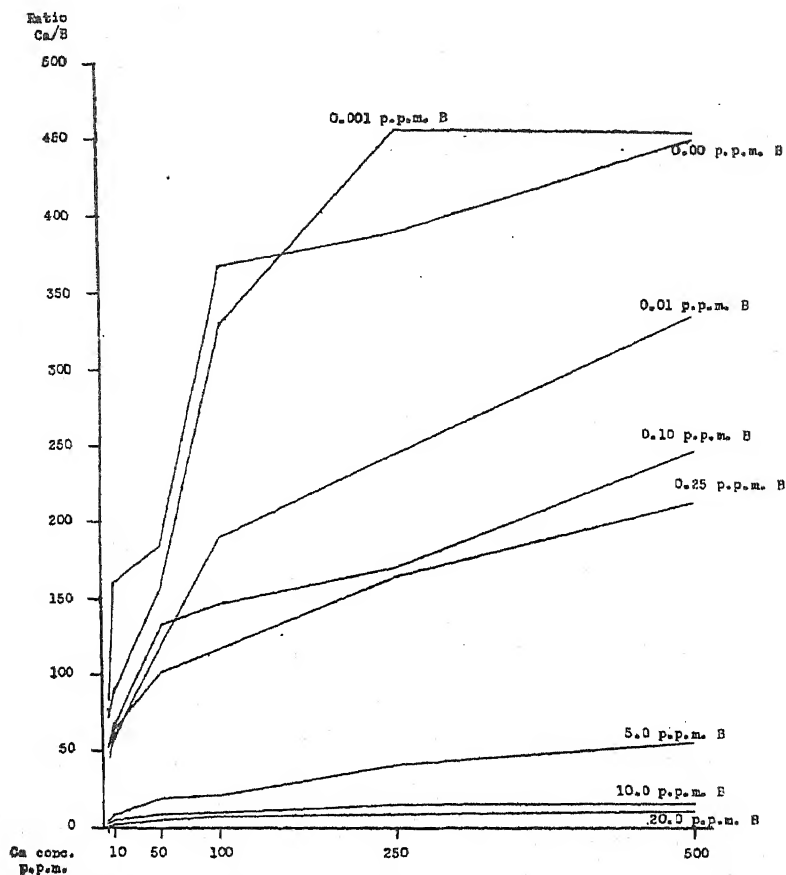
FIG. 7.—Ratios of total calcium to total boron of whole tops of corn plants grown at different boron levels plotted against calcium concentration in the substrate.

The experimental results also show that, in general, at each nutrient level of calcium a relatively higher percentage of the total boron remains soluble in the corn plant at the three highest boron levels than at the lower boron concentrations. This is similar to results obtained by Marsh with corn in which the lowest and highest percentage of soluble boron occurred at deficient and toxic boron substrate levels respectively (18).

Within any given calcium level the tissue contents of soluble boron were smaller in the plants grown at the two lowest boron levels than at the higher boron levels (table 2). Plants containing the lowest soluble boron at any given calcium level showed evidence of typical boron deficiency symptoms. This suggests that in this ex-

TABLE No. 3

RATIOS OF DRY WEIGHT TISSUE CONTENTS OF TOTAL CALCIUM TO TOTAL BORON OF WHOLE TOPS OF CORN PLANTS OF SERIES I

Treatment		Total Ca / Total B	Treatment		Total Ca / Total B
Ca	B		Ca	B	
p. p. m.	p. p. m.		p. p.m.	p. p. m.	
5	0.0	84	100	0.0	368
5	0.001	72	100	0.001	331
5	0.01	48	100	0.01	191
5	0.10	51	100	0.10	148
5	0.25	45	100	0.25	118
5	5.0	3	100	5.0	22
5	10.0	25	100	10.0	10
5	20.0	1	100	20.0	9
10	0.0	163	250	0.0	389
10	0.001	92	250	0.001	455
10	0.01	63	250	0.01	244
10	0.10	67	250	0.10	170
10	0.25	64	250	0.25	164
10	5.0	0	250	5.0	43
10	10.0	4	250	10.0	16
10	20.0	1.6	250	20.0	11
50	0.0	185	500	0.0	446
50	0.001	156	500	0.001	449
50	0.01	120	500	0.01	323
50	0.10	133	500	0.10	246
50	0.25	103	500	0.25	217
50	5.0	20	500	5.0	56
50	10.0	9	500	10.0	16
50	20.0	6	500	20.0	13

periment a constant nutrient supply of boron ranging from 0.01 to 0.25 p.p.m. was required to allow corn plants to grow normally.

From the results with regard to total and soluble calcium presented in table 2, it is evident that total calcium content of the tissue is mainly determined by the calcium concentration in the substrate and is largely independent of the nutrient concentration of boron. At each of the three highest levels of calcium, plants grown with the highest boron treatment had a considerably higher total and soluble calcium content than plants grown with the other boron treatments. These differences appear large enough to have some significance but their determinative factors are not clear.

From the analytical data relating to calcium and boron contents of the plant, the ratios of total calcium to total boron for the plants grown at the different calcium and boron levels have been calculated and presented in table 3. In figure 7 are presented the same Ca/B ratios plotted against calcium concentrations of the substrate. It is evident from the ratios of table 3 that the tissue of boron deficient plants receiving nutrient treatments of 0.0 and 0.001 p.p.m. boron had the highest Ca/B ratios at any given nutrient level of calcium while the Ca/B ratios of the tissues of boron-toxic plants were the lowest. Tissues of normal plants receiving intermediate amounts of

boron had intermediate ratios. It appears from the results that the quantitative relationship between calcium and boron in the tissues greatly influences the metabolic activities of the corn plant. The qualitative observations made on the plants (figure 3 and 4) which exhibited symptoms of boron toxicity seem to be definitely associated with the calcium and boron contents of these plants. At 500.0 p.p.m. calcium and 20.0 p.p.m. boron, the boron toxicity symptons were considerably less severe than those of plants grown with the same nutrient level of boron but at lower calcium levels. They also had the highest Ca/B ratio, 13 to 1, while the plants grown at 5.0 p.p.m. calcium and 20.0 p.p.m. boron had a Ca/B ratio of only 1 to 1 (table 3.). Although the proportion of calcium to boron in the plants in the first case mentioned above (500.0 p.p.m., 20.0 p.p.m. boron) was probably not the optimum for normal plant metabolism, nevertheless it seemed to be associated in some way with less severe boron toxicity than was evident in the plants grown at the highest boron level at the lowest calcium concentration. These were the plants that had a very low Ca/B ratio (figure 3). It can also be seen from table 3 that as the boron increased at a given calcium level the Ca/B ratio decreased.

Results of Chemical Analyses of Tissue Fractions of Corn Plant Tops

The results of quantitative tests for total boron and total calcium of the tissue fractions of plants grown at low and high boron levels

TABLE No. 4

TOTAL BORON AND TOTAL CALCIUM OF TOP LEAVES, MIDDLE STEM, AND LOWER STEM OF CORN PLANTS OF SERIES I EXPRESSED AS MILLIGRAMS PER GRAM DRY TISSUE

Treatment		Top Leaves		Middle Stem		Low Stem	
Ca	B	Total B	Total Ca	Total B	Total Ca	Total B	Total Ca
p. p. m.	p. p. m.	mgm. p. g.	mgm. p. g.	mgm. p. g.	mgm. p. g.	mgm. p. g.	mgm. p. g
5	0.001	0.025	0.63	0.020	1.43	0.020	0.65
5	5.0	0.180	0.43	0.325	0.92	0.080	0.58
10	0.01	0.025	1.10	0.025	1.98	0.020	0.94
10	5.0	0.175	0.89	0.275	1.76	0.080	0.82
50	0.01	0.030	2.19	0.025	5.02	0.020	2.41
50	5.0	0.175	2.83	0.275	3.52	0.100	1.65
100	0.10	0.035	3.04	0.035	4.97	0.030	2.89
100	5.0	0.199	2.70	0.225	5.19	0.080	3.94
250	0.01	0.030	5.39	0.030	8.46	0.010	6.18
250	5.0	0.325	5.16	0.250	7.31	0.070	5.94
500	0.001	0.030	4.23	0.020	6.41	0.010	6.28
500	5.0	0.275	4.39	0.300	10.16	0.080	8.25

(0.001, 0.01, 0.10 and 5.0 p.p.m.) at each of the six calcium concentrations are presented in table 4. These were the plants which were divided into top leaves, middle and lower stem fractions. As was pointed out before, this preliminary study was carried out to obtain an idea as to the distribution of the total calcium and boron in these three different portions of the corn plant. An analysis of the data shows that in general at the lowest boron level and at each nutrient level of calcium, boron is more or less equally distributed among top leaves, middle stem, and lower stem, with the least amount found in the lower stem tissue. However, at the highest boron level, and at each calcium level, the boron content of the middle stem was greater than that of the top leaves with but one exception, and the lower stems had the least amount of boron at this boron level.

It is also evident from the results presented in table 4 that the tissue content of calcium was greater in the middle stem portion of the plant at any given nutrient level of calcium. The amount of calcium found in each tissue was directly related to the calcium concentration in the substrate, with one exception, and was not greatly influenced by the boron concentration of the nutrient solution, with two exceptions. This occurred in the case of plants grown with the culture solution containing both 5.0 p.p.m. boron and 500.0 p.p.m. calcium where the calcium content of the middle and lower stem was considerably more than the calcium content of the respective tissue fractions of plants grown at 500.0 p.p.m. calcium but with only 0.001 p.p.m. boron.

Table 4 shows also that a relatively large amount of calcium accumulated in the lower stem of the plant at any given calcium level. But at the highest calcium levels (250.0, 500.0 p.p.m.) the lower stem had considerably greater tissue content of calcium than the top leaves. Thus it is evident that at these two highest calcium concentrations a large proportion of calcium accumulated in the lower or older part of the plant and was not freely translocated to the upper or younger leaves of the plant.

SERIES II

Methods

Cultural Methods.—The second corn series was started on April 18, 1946 to study the response of plants under different environmental conditions from those under which the first experiment was conducted. As before, seeds of Rutgers Hybrid No. 2 field corn selected for uniform size and shape were used in this test. Five

seeds were planted in each pot, and after germination the best three seedlings were left to receive treatments. During the first week of growth all corn cultures were supplied with the same dilute culture solution used during the first week with Series I. No boron was added during this period in order that the seedlings might utilize the supply of this element in the seed as far as possible before treatments were started. During the second week of growth all cultures received the same nutrient solution except that boron was added at the rate of 0.01 p.p.m. At the end of the second week all cultures were vigorous and appeared healthy in all respects.

The calcium-boron nutrient treatments were started beginning with the third week and were maintained for 30 days. Before applying treatments to plants, the sand in each culture was flushed with distilled water, then flushed again with the nutrient solutions to be used according to the experimental plan. As before, nutrient solutions were applied by the continuous solution renewal method. The culture solutions used with the different nutrient levels of calcium and of boron as well as the experimental setup were the same as in Series I. The calcium chloride salt used in the culture solutions of this series was purified by recrystallization after it was found by analysis to contain small amounts of boron. At the end of the experimental period of 30 days, plants were harvested. As before, green and dry weights of the tops were obtained and frozen samples of the plants of each culture were prepared for analysis. In addition, one of the three plants from a culture in each of the six different calcium levels and receiving 0.0, 0.25, and 20.0 p.p.m. boron respectively, was divided into four fractions—top leaves, top stems, lower leaves, and lower stems—for determinations of total and soluble boron and calcium of each tissue.

In the above tissue fractions the ''top leaves'' consisted of the apical meristem and the leaves arising in its immediate proximity and extending above it. The ''top stems'' consisted of the culm tissue starting downward from the base of the apical meristem and including the uppermost two differentiated internodes without attached blades and sheaths. The ''lower leaves'' consisted of all the leaves of the stem below the level of the apical meristem. The ''lower stem'' fraction consisted of the nodes and internodes below the base of the ''top stems'', as defined above.

Chemical Methods.—The same procedure and methods used in the first series were followed for the determination of total and soluble boron and calcium in the tissues of this corn series. Because

of the lack of sufficient plant material in some of the tissue fractions it was necessary in those cases to ignite a 1–gram instead of a 2–gram sample and to make to 50 ml. instead of 100 ml. volume.

RESULTS

Character of Plants after Treatments.—The relative intensities of the external symptoms of metabolic disturbance due to deficient and toxic quantities of boron in the nutrient media are represented by the proportion of the shaded areas in the blocks of the diagram of figure 8. If this diagram is compared with figure 1 a striking difference will be noted in the response of the plants to the low and high boron treatments at the various calcium concentrations. Symptoms of boron deficiency appeared first in the plants at the highest nutrient calcium concentrations, 19 days after treatment. At the end of the experimental period all plants at the two lowest boron levels of 0.0 and 0.001 p.p.m. showed boron deficiency symptoms, except those grown at the two lowest calcium levels. The boron deficiency symptoms increased in severity progressively as the calcium concentration of the substrate increased, except that somewhat more intense boron deficiency symptoms were detected at 250.0 p.p.m. calcium than at 500.0 p.p.m. calcium concentration.
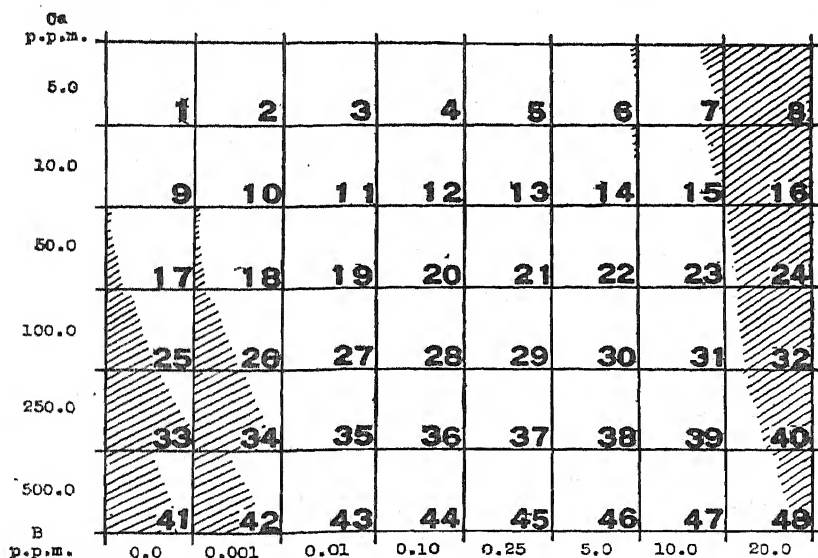

FIG. 8.—Diagram showing calcium and boron treatments and general experimental setup of Series II. Proportionate amounts of shading in the blocks indicate approximate relative severity of boron deficiency symptoms (left) and boron toxicity symptoms (right).

The growing points of boron deficient plants showed a brownish color as compared to the creamy-white color of growing points of healthy plants. The roots were brown and the root systems were less extensive than those of plants receiving adequate amounts of boron. As before, the boron deficiency symptoms of the roots were most severe at the low calcium levels. This was probably due to combination of both boron and calcium deficiency.

In this series, in contrast to the first series (figures 1, 8), there was virtually no indication of boron toxicity at 5.0 p.p.m. boron. With the exception of the plants at the two calcium levels of 5.0 and 10.0 p.p.m., where boron toxicity was only slight, the plants at other calcium levels did not show evidence of boron toxicity at 10.0 p.p.m. boron. However, as is evident from figure 8, injury due to boron toxicity occurred at 20.0 p.p.m. boron at all calcium levels but decreased in severity with increasing calcium concentration in the substrate. As in Series I, boron-toxic plants were characterized by a yellow-green color of the top leaves and by brown, dead margins and tips of the older or lower leaves. The roots of these plants were mostly brown and less abundant, especially at the two lowest calcium levels, as compared with the long, silvery-white and healthy roots of the plants receiving boron ranging between 0.01 to 0.25 p.p.m. Roots of plants receiving 5.0 and 10.0 p.p.m. boron showed slight boron injury as evidenced by their light brown color as compared with the silvery-white color of roots of plants supplied with lower concentrations of boron.

In the plants of this series the severity of calcium deficiency symptoms was considerably more pronounced in plants grown at 5.0 and 10.0 p.p.m. calcium than in the same treatments in Series I planted in the fall season. Plants were most severely injured at the lowest calcium level (5.0 p.p.m.) and were stunted considerably in vegetative growth (30). In some of the plants the young leaves failed to unroll properly and the tips of these cylinders of unrolled leaf tissue were dead or dying. The root systems of these calcium deficient plants were less extensive and were slightly browner than the roots of plants receiving larger amounts of calcium in the substrate. This probably indicates an early maturity of the tissues.

Results of Chemical Analyses.—The results of quantitative tests for total and soluble boron and calcium are presented in table 5. An examination of these data clearly demonstrates that the total and soluble boron in the corn plant is largely a direct function of the boron concentration in the substrate and is only slightly in-

TABLE No. 5

TOTAL AND SOLUBLE CALCIUM AND BORON IN MILLIGRAMS PER GRAM
DRY TISSUE OF WHOLE TOPS OF CORN PLANTS OF SERIES II

Treatment		Total	Soluble	Soluble B	Total	Soluble
Ca	B	B	B	Per cent of Total	Ca	Ca
p. p. m.	p. p. m.	mgm. p. g.	mgm. p. g.	Per cent	mgm. p. g.	mgm. p. g.
5	0.0	0.020	0.007	30.0	0.61	0.30
5	0.001	0.030	0.013	43.3	0.72	0.30
5	0.01	0.040	0.022	55.0	0.61	0.31
5	0.10	0.045	0.026	57.7	0.78	0.39
5	0.25	0.045	0.025	55.5	0.66	0.25
5	5.0	0.175	0.132	75.3	0.63	0.31
5	10.0	0.424	0.358	84.7	0.69	0.22
5	20.0	0.624	0.529	84.7	0.54	0.18
10	0.0	0.020	0.006	20.0	0.97	0.55
10	0.001	0.025	0.013	52.0	0.81	0.51
10	0.01	0.030	0.015	53.3	0.86	0.53
10	0.10	0.035	0.023	65.7	0.92	0.58
10	0.25	0.035	0.025	71.4	0.92	0.42
10	5.0	0.175	0.147	84.0	0.78	0.39
10	10.0	0.325	0.265	81.5	0.88	0.54
10	20.0	0.625	0.536	85.7	0.77	0.45
50	0.0	0.025	0.011	44.4	2.87	1.96
50	0.001	0.025	0.013	52.0	2.33	1.62
50	0.01	0.030	0.017	56.6	2.64	1.72
50	0.10	0.035	0.023	65.7	2.58	1.83
50	0.25	0.040	0.025	62.5	2.11	1.24
50	5.0	0.137	0.119	86.8	2.34	1.80
50	10.0	0.325	0.288	88.8	2.56	1.96
50	20.0	0.625	0.526	76.4	2.36	1.31
100	0.0	0.020	0.009	45.0	4.49	3.33
100	0.001	0.020	0.010	50.0	4.33	3.19
100	0.01	0.025	0.012	48.0	3.69	2.78
100	0.10	0.029	0.020	68.9	3.39	2.15
100	0.25	0.040	0.022	55.0	4.00	3.18
100	5.0	0.137	0.102	74.4	3.77	2.52
100	10.0	0.325	0.267	82.1	4.36	3.37
100	20.0	0.749	0.650	86.7	4.42	3.26
250	0.0	0.020	0.009	45.0	5.39	2.76
250	0.001	0.025	0.012	40.0	5.06	3.36
250	0.01	0.025	0.015	60.0	4.82	3.49
250	0.10	0.030	0.019	63.3	3.95	3.04
250	0.25	0.040	0.022	55.0	4.97	3.70
250	5.0	0.137	0.111	81.0	4.70	3.40
250	10.0	0.325	0.263	80.9	5.60	4.32
250	20.0	0.750	0.608	81.0	5.11	3.58
500	0.0	0.025	0.010	40.0	5.56	4.62
500	0.001	0.025	0.010	40.0	5.27	3.84
500	0.01	0.030	0.019	63.3	5.76	3.38
500	0.10	0.040	0.021	52.5	6.07	3.38
500	0.25	0.040	0.025	62.5	5.15	4.04
500	5.0	0.175	0.139	79.4	5.59	3.92
500	10.0	0.325	0.255	78.4	5.55	4.13
500	20.0	0.625	0.447	71.5	9.25	5.84

fluenced by the calcium level. It is also evident from table 5 that, as was found in Series I, the soluble boron within the plant was somewhat less at the highest calcium level (500.0 p.p.m.) than at all of the lower nutrient levels of calcium. As in the first series, the data also show that in most cases a relatively higher percentage of the total boron remains soluble in the corn plant at the high boron levels than at lower nutrient levels of boron. However, the data fail to explain the qualitative observations made earlier with

TABLE No. 6

RATIOS OF DRY WEIGHT TISSUE CONTENTS OF TOTAL CALCIUM TO TOTAL
BORON OF WHOLE TOPS OF CORN PLANTS OF SERIES II

| Treatment | | Total Ca | Treatment | | Total Ca |
Ca	B	Total B	Ca	B	Total B
p. p. m.	p. p. m.		p. p. m.	p. p. m.	
5	0.0	31	100	0.0	226
5	0.001	24	100	0.001	218
5	0.01	15	100	0.01	148
5	0.10	17	100	0.10	119
5	0.25	15	100	0.25	103
5	5.0	3	100	5.0	27
5	10.0	2	100	10.0	13
5	20.0	0.9	100	20.0	6
10	0.0	49	250	0.0	271
10	0.001	33	250	0.001	203
10	0.01	29	250	0.01	194
10	0.10	26	250	0.10	132
10	0.25	26	250	0.25	125
10	5.0	4	250	5.0	34
10	10.0	3	250	10.0	17
10	20.0	1	250	20.0	7
50	0.0	115	500	0.0	223
50	0.001	94	500	0.001	212
50	0.01	88	500	0.01	193
50	0.10	74	500	0.10	152
50	0.25	53	500	0.25	129
50	5.0	17	500	5.0	32
50	10.0	8	500	10.0	17
50	20.0	6	500	20.0	15

regard to boron deficiency, that is, that this injury is intensified
with increasing concentrations of calcium in the substrate, nor does
it satisfactorily explain why boron toxicity decreased with increasing
concentrations of calcium in the substrate. In tomato plants (4,37),
the qualitative observations are in agreement with the quantitative
data with regard to boron deficient and boron toxic plants. An
explanation for this lack of agreement in the corn plant will be given
later when the quantitative relationship between calcium and boron
in the plant is considered, together with the analytical data with
respect to total and soluble boron of the fractionated portions of
the plant.

From the results presented with regard to total and soluble
calcium of the plant, it is evident that the same trend follows as
with the results obtained for the first series, that is, that the calcium
content in the tissues is largely determined by the calcium concentra-
tion of the growth medium and is not greatly influenced by boron,
with one principal exception, namely, in the case of plants grown
at 500.0 p.p.m. calcium and 20.0 p.p.m. boron where total and soluble
calcium content was much higher as compared with that of plants
grown at the same calcium level but different boron concentrations.
With the exception mentioned above, similar results were obtained

TABLE No. 7

TOTAL AND SOLUBLE BORON AND CALCIUM IN MILLIGRAMS PER GRAM DRY TISSUE OF *TOP LEAVES* OF CORN PLANTS OF SERIES II

Treatment		Total	Soluble	Total	Soluble
Ca	B	B	B	Ca	Ca
p. p. m.	p. p. m.	mgm. p. g.	mgm. p. g.	mgm. p. g.	mgm. p. g.
5	0.0	0.040	0.014	0.22	0.003
10	0.0	0.025	0.012	0.76	0.04
50	0.0	0.022	0.012	1.33	0.38
100	0.0	0.025	0.005	2.47	0.58
250	0.0	0.012	0.001	3.04	2.17
500	0.0	0.019	0.004	4.46	2.87
5	0.25	0.044	0.030	0.52	0.03
10	0.25	0.044	0.028	0.78	0.37
50	0.25	0.045	0.028	2.45	1.62
100	0.25	0.045	0.026	4.04	2.82
250	0.25	0.045	0.028	3.81	2.06
500	0.25	0.045	0.031	3.05	1.85
5	20.0	0.624	0.437	0.25	0.09
10	20.0	0.624	0.391	0.56	0.27
50	20.0	0.624	0.295	1.85	0.95
100	20.0	0.874	0.727	2.33	1.38
250	20.0	0.874	0.696	2.95	1.74
500	20.0	0.873	0.743	4.02	2.45

by Warington working with *Vicia faba* (47), Reeve (36), Brennan (4) working with tomato, and Reeve and Shive (37) working with both corn and tomato plants.

Again the ratios of total calcium to total boron in the plants were calculated and are presented in table 6. As in Series I, somewhat similar differences in ratios were obtained. It is evident from table 6 that, in general, at a given boron level as the calcium concentration in the substrate increase, the ratio of Ca/B increases also, while at a given calcium level as the boron in the substrate increases, the ratio decreases. Plants showing boron deficient symptoms had rather high Ca/B ratios especially at the higher calcium concentrations in the substrate, whereas plants with relatively intense boron-toxic symptoms had a very low ratio at the low calcium concentrations as compared with the intermediate ratios of healthy plants. As with the first corn series, the ratios presented indicate that a certain balance must exist between the calcium and boron content of the corn tissue to fulfill the requirements of healthy individuals. The fact that boron deficient and boron toxic plants had a high and low Ca/B ratio, respectively, points to the importance of the relationship between these two elements within the plant. The data of table 6 show that at the highest boron level (20.0 p.p.m.) as the calcium concentration of the substrate was increased the Ca/B ratio increased, as was to be expected, and is considerably higher at the last four calcium levels (50.0, 100.0, 250.0, 500.0 p.p.m. calcium) than at the first two calcium levels, respectively, (5.0,

TOTAL AND SOLUBLE BORON AND CALCIUM IN MILLIGRAMS PER GRAM
DRY TISSUE OF *LOW LEAVES* OF CORN PLANTS OF SERIES II

Treatment		Total	Soluble	Total	Soluble
Ca	B	B	B	Ca	Ca
p. p. m.	p. p. m.	mgm. p. g.	mgm. p. g.	mgm. p. g.	mgm. p. g.
5	0.0	0.030	.005	1.16	0.48
10	0.0	0.025	.015	1.43	0.62
50	0.0	0.025	.014	3.80	1.63
100	0.0	0.025	.008	4.83	3.04
250	0.0	0.022	.012	7.61	5.24
500	0.0	0.040	.023	9.13	6.88
5	0.25	0.056	.037	0.96	0.61
10	0.25	0.044	.025	1.35	0.55
50	0.25	0.045	.025	4.59	3.41
100	0.25	0.045	.027	5.48	4.03
250	0.25	0.045	.028	8.72	6.25
500	0.25	0.045	.028	7.77	5.81
5	20.0	1.750	1.585	0.91	0.52
10	20.0	0.874	.737	0.88	0.49
50	20.0	0.874	.455	4.01	2.46
100	20.0	1.130	.970	4.67	3.32
250	20.0	1.500	1.342	7.52	5.73
500	20.0	1.500	1.335	10.33	8.12

10.0 p.p.m. calcium). It was at these last four calcium concentrations that the plants showed a decrease in the intensity of injury due to boron toxicity, as can be observed in figure 8. The less toxic boron plants had a Ca/B ratio of 7 and 15 respectively as compared with ratios of 0.9 and 1 respectively of those plants showing much more boron toxicity. Thus it is apparent that there seems to exist a direct association between the appearance of boron deficiency and toxicity symptoms in the corn plant and the quantitative relationships of calcium and boron within the plant.

Results of Chemical Analyses of Tissue Fractions.—The analytical results of quantitative tests for total and soluble boron and total and soluble calcium of the tissues of the fractionated plants are presented in tables 7, 8, 9, and 10. The results obtained bring out several interesting points with regard to total and soluble boron content of the various tissues at the different boron levels. The tables show that, in general, at the lowest boron concentration of the substrate there was a greater accumulation of total and soluble boron in the older tissues of the plant (top stems, low stems, and low leaves) than in the top leaves and these differences were particularly marked at the highest nutrient levels of calcium. At the lowest nutrient level of boron the soluble boron contents of the top leaves at the three highest calcium levels (100.0, 250.0, and 500.0 p.p.m.) are relatively low as compared with the values at the three lower calcium levels. These data are in agreement with the qualita-

TABLE No. 9

TOTAL AND SOLUBLE BORON AND CALCIUM IN MILLIGRAMS PER GRAM
DRY TISSUE OF *TOP STEMS* OF CORN OF SERIES II

Treatment		Total	Soluble	Total	Soluble
Ca	B	B	B	Ca	Ca
p. p. m.	p. p. m.	mgm. p. g.	mgm. p. g.	mgm. p. g.	mgm. p. g.
5	0.0	....	....	....	....
10	0.0	0.030	0.011	0.75	0.41
50	0.0	0.030	0.013	1.42	0.99
100	0.0	0.030	0.009	1.78	1.13
250	0.0	0.040	0.017	2.93	1.88
500	0.0	0.050	0.016	3.51	2.59
5	0.25	0.050	0.023	0.44	0.17
10	0.25	0.045	0.027	0.50	0.26
50	0.25	0.030	0.012	1.34	0.85
100	0.25	0.040	0.013	2.00	1.36
250	0.25	0.040	0.015	2.45	1.68
500	0.25	0.050	0.019	4.55	1.64
5	20.0	0.125	0.087	0.26	0.01
10	20.0	0.350	0.307	0.56	0.11
50	20.0	0.299	0.261	1.89	1.32
100	20.0	0.225	0.202	2.73	1.96
250	20.0	0.274	0.218	7.01	5.50
500	20.0	0.225	0.178	6.44	4.99

tive observations made at the time of harvest, when it was noted
that the boron-deficiency symptoms of the top leaves of the corn
plant were most severe at the three highest calcium levels. How-
ever, it should be mentioned that the boron deficient injury was
more pronounced in the plants supplied with 250.0 p.p.m. calcium
than at either of the other two highest calcium levels. Thus, it is
evident, that symptoms of boron deficiency are associated with the
presence of small amounts of boron in the top portions of boron
deficient plants as compared with plants receiving adequate amounts
of boron (9). It appears from the results presented that at low
boron concentration, a large proportion of boron accumulates in the
older tissues of the plants and is not freely translocated to the top
leaves where synthetic activity is highest. The fact that a large
part of the total and soluble boron in plants to which boron was
not intentionally supplied accumulated in the lower or older tissues
instead of in the top leaves was probably responsible for the lack
of agreement between the qualitative observations of boron deficient
plants made at the time of harvest and quantitative results of the
same plants when a composite sample of the whole top was analyzed
for total and soluble boron. Unquestionably the low total and soluble
boron content of the top leaves was masked by the presence of tissues
containing high total and soluble boron (top stems, low leaves, and
low stems) in the composite sample and, therefore, the qualitative
observations of the boron deficient plants were not confirmed by
quantitative analysis of whole tops. This indicates that the values

TABLE No. 10

TOTAL AND SOLUBLE BORON AND CALCIUM IN MILLIGRAMS PER GRAM
DRY TISSUE OF *LOW STEMS* OF CORN PLANTS OF SERIES II

Treatment		Total	Soluble	Total	Soluble
Ca	B	B	B	Ca	Ca
p. p. m.	p. p. m.	mgm. p. g.	mgm. p. g.	mgm. p. g.	mgm. p. g.
5	0.0	0.020	0.004	0.29	0.13
10	0.0	0.045	0.017	0.54	0.30
50	0.0	0.045	0.030	1.26	0.89
100	0.0	0.020	0.008	3.36	2.86
250	0.0	0.030	0.013	4.05	3.22
500	0.0	0.040	0.012	6.86	5.58
5	0.25	0.022	0.008	0.28	0.15
10	0.25	0.022	0.008	0.35	0.21
50	0.25	0.034	0.014	1.56	1.19
100	0.25	0.045	0.024	2.83	2.29
250	0.25	0.045	0.020	3.46	2.66
500	0.25	0.040	0.016	5.54	4.20
5	20.0	0.175	0.161	0.29	0.03
10	20.0	0.350	0.313	0.34	0.15
50	20.0	0.349	0.309	2.01	1.46
100	20.0	0.274	0.246	2.41	1.92
250	20.0	0.349	0.304	6.99	5.97
500	20.0	0.350	0.302	13.33	11.29

of total and soluble boron contents of boron deficient plants as a
whole do not provide reliable criteria of the concentration present
in any given tissue.

The plants of the cultures supplied with 0.25 p.p.m. boron
showed a much more equal distribution of this element throughout
the various tissues than plants supplied with no boron or with very
high nutrient levels of this element. At the nutrient level of 0.25
p.p.m. boron there was relatively little variation in the total boron
contents of any given tissue fraction of plants grown with different
calcium levels. At the same nutrient level of boron (0.25 p.p.m.)
the soluble boron was higher in the top leaves and lower leaves
than in the top and lower stems of the plants at any given calcium
level, except in one case. This might be expected, since the leaves
are more active than the stems in metabolic processes in which
boron must take part.

The analytical data for total and soluble boron of all the cultures
supplied with 20.0 p.p.m. boron show that the distribution of boron
in the plant followed a definite course (19). The greatest amount
of total and soluble boron accumulated in the lower leaves, while a
considerably lesser amount was found in the top leaves. Low stems
had a higher boron content than top stems. The qualitative observa-
tions made in regard to boron toxicity symptoms of plants grown at
the highest boron level are in agreement with the quantitative data

TABLE No. 11

RATIOS OF DRY WEIGHT TISSUE CONTENTS OF TOTAL CALCIUM TO TOTAL
BORON IN THE VARIOUS FRACTIONS OF THE CORN PLANTS OF SERIES II

Treatment		Top Leaves	Low Leaves	Top Stems	Low Stems
		$\dfrac{\text{Total Ca}}{\text{Total B}}$	$\dfrac{\text{Total Ca}}{\text{Total B}}$	$\dfrac{\text{Total Ca}}{\text{Total B}}$	$\dfrac{\text{Total Ca}}{\text{Total B}}$
Ca	B				
p. p. m.	p. p. m.				
5	0.0	5	38	....	15
10	0.0	31	57	25	12
50	0.0	59	153	47	28
100	0.0	99	194	60	109
250	0.0	245	340	73	135
500	0.0	224	229	70	172
5	0.25	12	17	9	13
10	0.25	18	31	11	16
50	0.25	55	102	45	46
100	0.25	90	123	50	65
250	0.25	85	194	62	77
500	0.25	68	173	91	139
5	20.0	0.4	0.5	2	2
10	20.0	0.9	0.7	2	1
50	20.0	3.0	5.0	6	6
100	20.0	3.0	4.0	12	9
250	20.0	3.0	5.0	26	20
500	20.0	5.0	7.0	29	38

presented. The higher concentration of boron in the lower leaves
paralleled the presence of boron toxic effects as evidenced by the
brown and dead margins and tips of the lower leaves of the plants.
Similar results were obtained by Eaton (8) and Purvis (34) due
to a great accumulation of boron in the lower leaves of the plants.

The data further show that the total and soluble boron content
of the various tissues depended largely upon the boron concentra-
tion of the substrate. However, in some cases the relative calcium
concentration of the substrate modified the accumulation of this
element within the plant at a given boron level, the greatest modifica-
tion occurring at the highest boron level. As a general rule, the
leaves were more sensitive to boron changes in the substrate than
were either the top or low stems. Parks, Lyons, and Hood (31)
reported that as boron supply in the substrate increased, the con-
centration of boron in the leaves increased significantly.

Calcium Content of Plant Tissues.—The analytical evidence
gathered with reference to the calcium content of the various tissues
of the corn plant shows that its distribution is regular and definite.
The greatest amount of total and soluble calcium was found in the
leaves of the plant, the lower leaves having the largest amount of
this element (45). In general, the lower stems had a much higher
calcium content than the top stems. The fact that much more total

and soluble calcium was found in the lower leaves and lower stems suggests that most of it was tied up in this lower portion of the plant and, therefore, was not freely translocated to the younger tissue where meristematic activity is highest. As a result of this condition the severe calcium deficiency symptoms of the cultures grown at 5.0 p.p.m. calcium and less marked deficiency symptoms at 10.0 p.p.m. calcium can be explained. It is interesting to note from table 7 that the top leaves of those plants grown at these two lowest calcium levels without boron contained considerably less soluble calcium than the top leaves of those plants grown at the same calcium levels but having boron in the substrate. The total calcium of the top leaves of the calcium deficient plants (5.0, 10.0 p.p.m. calcium) receiving no boron and 20.0 p.p.m. boron respectively at each of the two lowest calcium levels are comparable. There were only little differences between the total calcium contents of the top leaves of the plants grown at the lowest boron level (0.0 p.p.m.) and the
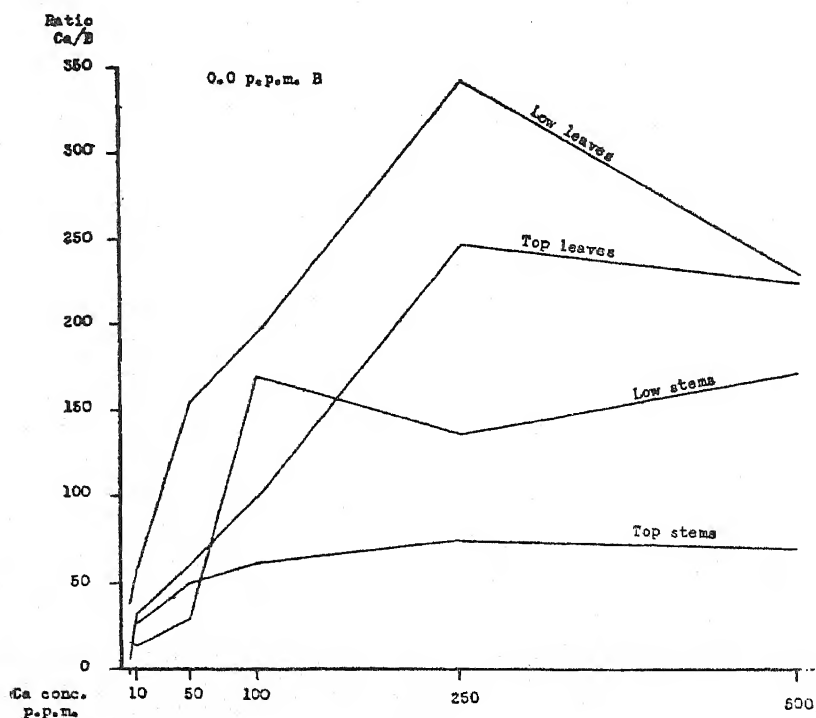
FIG. 9.—Ratios of total calcium to total boron in each fraction of corn plants grown at 0.0 p.p.m. boron plotted against calcium concentration in the substrate.

highest boron level (20.0 p.p.m.) at either of the two lowest nutrient levels of calcium respectively (5.0, 10.0 p.p.m.). This, however, was not true in the case of the soluble calcium content of these same tissues; the soluble calcium content being considerably greater in the calcium deficient plants grown at the highest boron level. These differences may be associated with the soluble boron content of the tissues of these calcium deficient plants. Where the soluble calcium content of the tissue was greatest, it was found that the soluble boron content was also the greatest. In other words, the soluble calcium in the top leaves of these calcium deficient plants is not so much a function of the total calcium as it is of the soluble boron. This relation described above did not hold true in the case of the other plant fractions. The same relation between the tissue content of soluble calcium and soluble boron has been obtained by Marsh and Shive (17) for whole tops of calcium deficient corn plants, by Lowenhaupt with the sunflower (16), and by Lorenz with garden beet (15). In general, at a given boron level as the calcium concentration of the substrate increased, there resulted an increased accumulation of total and soluble calcium in each of the various fractionated portions of the plant (47). In general, at a given calcium level, variations in the boron concentrations of the substrate did not influence greatly the calcium content of the tissue of the various plant fractions, except at the two highest calcium levels in the case of the top stems and lower stems where the values of total and soluble calcium were very high at the highest boron level.

From the analytical data pertaining to the calcium and boron content of the various tissues of the plants grown at various nutrient treatments, the Ca/B ratios were calculated in a manner similar to that previously used to determine Ca/B ratios for the whole tops. These ratios are presented in table 11. It is evident that plants showing boron deficient symptoms had the highest Ca/B ratios in the various fractionated portions while those giving evidence of boron toxicity and calcium deficiency had very low Ca/B ratios. On the other hand, the plants which did not show any injury and which were apparently healthy had intermediate ratios. The plants which had the greatest green and dry weights, those supplied with 250.0 p.p.m. calcium and 0.25 p.p.m. boron, and which were in all respects healthy, had a ratio of Ca/B of 125 as calculated from the composite sample of the whole tops (table 6). The Ca/B ratios of the individual tissue fractions of the plants grown with the same nutrient treatments were as follows: top leaves 85; low leaves 194; top stem 62; and low stem 77 (table 11). As was to be expected,
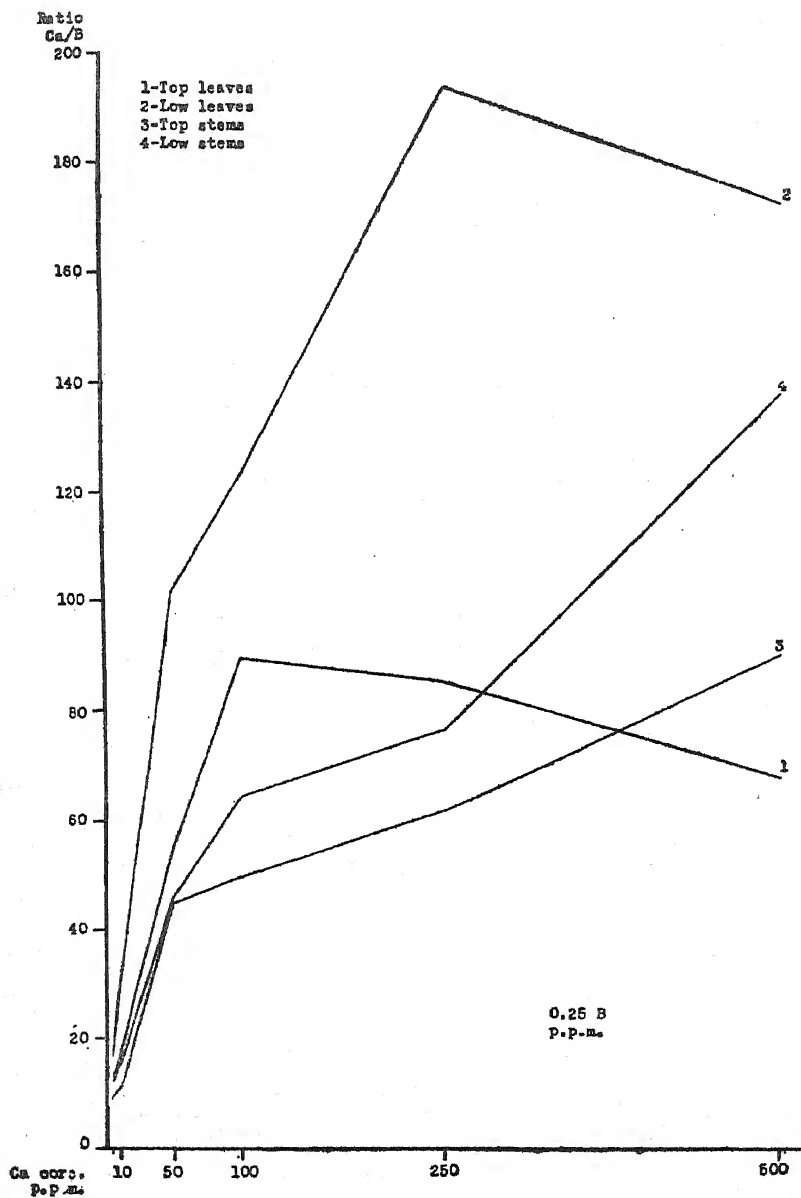
FIG. 10.—Ratios of total calcium to total boron in each fraction of corn plants grown at 0.25 p.p.m. boron plotted against calcium concentration in the substrate.

because of the greater accumulation of calcium and boron in the older tissues of the plant, the Ca/B ratios of the lower leaves were higher than those of the top leaves in all but one case. The same trend followed with regard to the Ca/B ratios of the lower stem and the top stem fractions except that at the highest boron concentration there was not much difference between the Ca/B ratios of the top and the low stem tissues respectively at any nutrient level of calcium except the highest (500.0 p.p.m.).

As can be seen from table 11 and figures 9, 10, 11, 12 and 13, as the calcium increased at a given boron level, the Ca/B ratio increased in the majority of cases. On the other hand, as the boron increased at a given calcium level, the ratio decreased.

From the analytical results and Ca/B ratios presented for both series of corn, it is evident that a certain relationship exists between calcium and boron which affects the metabolism of the corn plant, but which, however, is not so striking as with the tomato (4, 37). The quantitative results obtained in the second corn series for the total and soluble boron and calcium contents of the composite samples did not confirm the qualitative observations made, namely, that the boron deficiency symptoms were increasingly severe with increasing calcium concentration of the substrate. However, when a
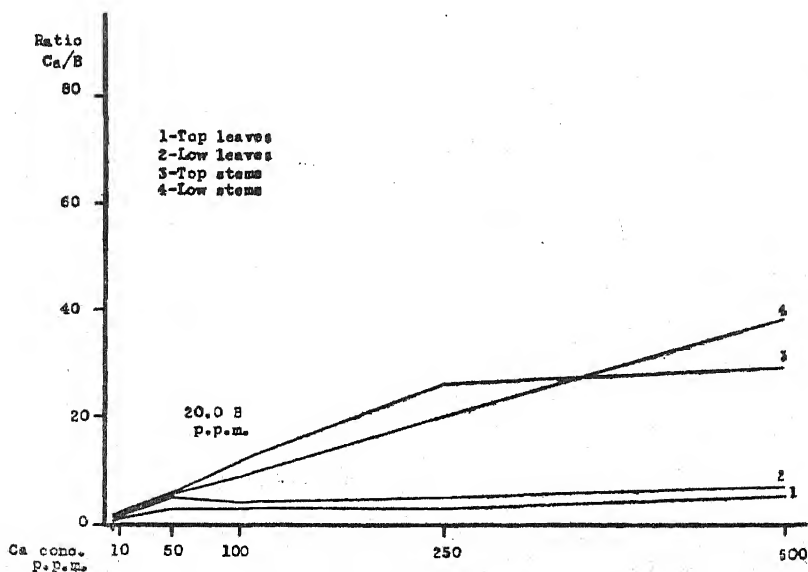

FIG. 11.—Ratios of total calcium to total boron in each fraction of corn plants grown at 20.0 p.p.m. boron plotted against calcium concentration in the substrate.

representative plant from the culture supplied with no boron at the highest calcium levels was fractionated, the analytical results for total and soluble boron for the top leaves were in agreement with the qualitative observations made on the tops of these boron deficient plants. Since a considerable amount of total and soluble boron of these boron deficient plants was found in the older tissues as compared with the top leaves, probably this was the reason why the analytical data of the composite samples did not corroborate the qualitative observations. The higher total and soluble boron of these fractions masked the small amount of total and soluble boron of the top leaves when all the tissues were mixed to take the composite sample, and, therefore, little or no difference was evident between boron contents of whole top samples of different nutrient treatments. The results further suggest that there is probably little
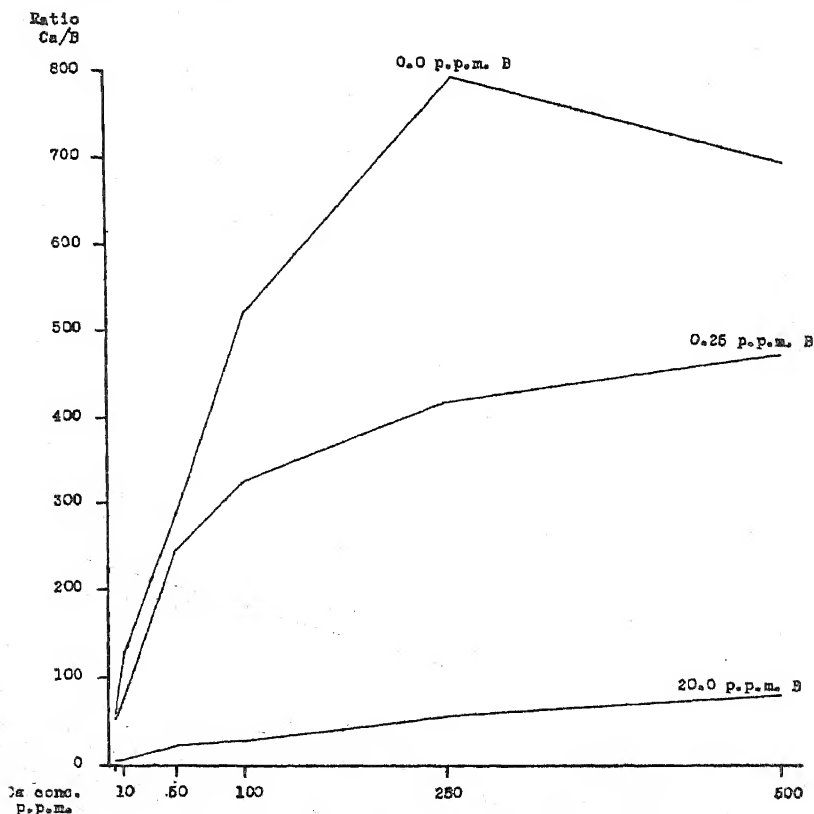

FIG. 12.—Ratios of the averages of tissue contents of total calcium to total boron of the four different plant fractions of corn plants grown at nutrient levels of 0.0, 0.25, and 20.0 p.p.m.

if any translocation of soluble boron from the older tissues of the boron deficient plants to the top leaves where meristematic activity is at its maximum. It should be mentioned at this point that it took a considerable time for the boron deficiency symptoms to appear in these cultures in both series. However, it took less time than in the similar cultures grown in the fall season (46). The fact that the boron requirements for corn are rather low (18) and that a relatively large proportion of this element is in the soluble state in the plant tissue may explain the late appearance of the injury due to boron deficiency in the top leaves and roots. This also suggests why corn plants made fairly good growth up to the time when the deficiency symptoms appeared. It is probable that the late appearance of boron deficiency symptoms in plants, to which boron was not intentionally supplied, was due in part to the presence of boron as impurities in the salts used. In fact, it was found necessary to repurify one of the salts, namely, $CaCl_2$ for the second series, by recrystalization.

As was pointed out previously, the Ca/B ratios of the boron deficient plants were high. Plants that received no boron in the substrate and which were supplied with calcium concentrations of 5.0, 10.0, and 50.0 p.p.m. had a much lower Ca/B ratio than those
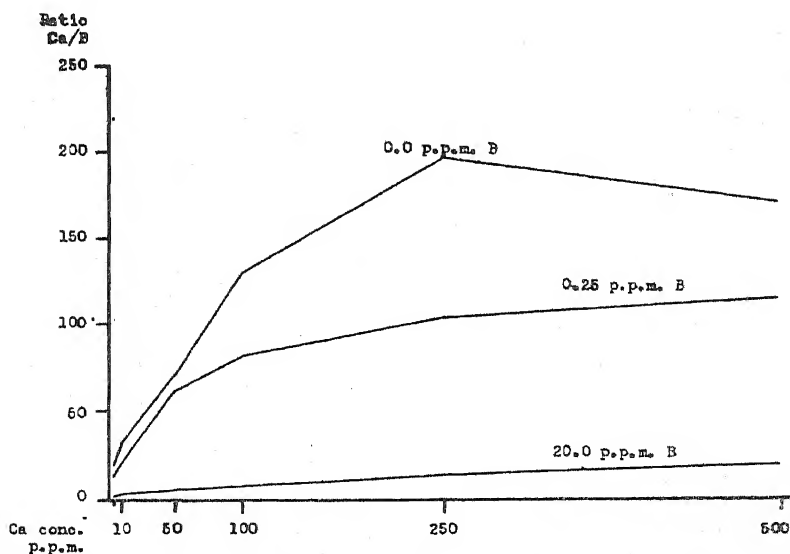

FIG. 13.—Ratios of total calcium to total boron of whole tops of corn plants grown at boron levels of 0.0, 0.25, and 20.0 p.p.m. plotted against calcium concentration in the substrate.

at the highest calcium nutrient levels and showed either slight boron deficiency symptoms or no symptoms at all. It appears that the combination of high calcium and deficient boron in the substrate upsets the normal metabolism of the plant more seriously than a combination of low calcium and deficient boron.

Neither the quantitative data for total and soluble boron of the composite nor the fractionated portions of the plant confirmed completely the qualitative observations made with regard to a decrease of boron toxicity in the plants supplied with 20.0 p.p.m. boron as the calcium concentration of the substrate increased. On the other hand, the calculated Ca/B ratios for the composite and fractionated portions (table 6 and table 11) of the plants furnish some interesting information. It is in the quantitative relationship between the calcium and boron within the plant that the decrease in the severity of boron toxic symptoms can be associated. The plants that showed relatively mild boron toxicity symptoms, which were the plants grown with the highest calcium concentrations of the substrate, had higher Ca/B ratios than those showing more severe boron toxicity symptoms. This indicates that a combination of high calcium and high boron in the substrate tends to favor a more normal metabolism of the plant than a combination of low calcium and high boron, which brings about a disturbance in the metabolic activities of the plant.

The plants grown in the substrate containing 0.01 to 0.25 p.p.m. boron were devoid of any boron deficiency or boron toxicity symptoms, thus indicating that their metabolism was normal. These plants had Ca/B ratios which were intermediate between those of boron-deficient and boron-toxic plants.

The quantitative results presented in this report for total and soluble calcium in the corn plant definitely show that the quantitative relationship between these two elements within the plant plays a very important role in the metabolic activities of the plant. Moreover, the data show that a certain balance between calcium and boron must exist within the plant to fulfill the requirements for normal growth and development of this monocotyledon.

SUMMARY

Corn plants were grown in sand culture using the continuous flow method with six different kinds of nutrient solutions which contained different calcium and boron concentrations. A qualitative and quantitative study was made of the response of the plants to the different calcium and boron levels. The results may be summarized as follows:

1. Plants showing symptoms of boron deficiency were found by chemical analyses to have a low content of total and soluble boron and those showing symptoms of boron toxicity a very high content of total and soluble boron.

2. Boron deficient plants were characterized by the appearance of elongated, white transparent stripes in the newly formed leaves. The growth of boron toxic plants was stunted, their top leaves yellow or yellow-green and margins and tips of lower leaves brown and dead.

3. Composite samples of the whole tops of plants showed by analysis that both total boron and soluble boron were largely independent of the calcium concentration in the substrate, except that soluble boron content of the plants grown with the highest boron level (20.0 p.p.m.) was less when supplied with nutrient levels of 500.0 p.p.m. calcium than with the lower calcium levels.

4. The accumulation of calcium in the composite tissue samples of the tops was found to be largely determined by the calcium concentration in the substrate, except that at the highest nutrient level of boron (20.0 p.p.m.) the calcium content of the tissue of plants grown with high nutrient levels of calcium (100.0, 250.0, 500.0 p.p.m.) was higher than those of plants grown at lower nutrient levels of boron.

5. Boron deficiency symptoms increased in severity with increasing calcium concentration of the substrate throughout the range of the four highest nutrient levels of calcium, except that somewhat more intense boron deficiency symptoms were detected at 250.0 p.p.m. calcium than at 500.0 p.p.m. calcium concentration. Top leaves of boron deficient plants grown at 0.0 p.p.m. boron and which showed boron deficiency symptoms had, in general, lower total and soluble boron content than top leaves of plants grown at the same boron

level but at the two lowest calcium levels which did not exhibit boron deficiency symptoms. The older tissues of these plants showing external boron deficiency symptoms had a considerably higher total and soluble boron content than the younger tissues (top leaves).

6. The results of this study indicate that there is a limited rate of translocation of boron from the lower portions to the top leaves of boron deficient plants.

7. Boron toxicity decreased in severity with increasing calcium concentration of the substrate.

8. Increasing the concentration of boron in the culture solution resulted in a marked increase of total and soluble boron in all tissues of the plant.

9. There was a greater accumulation of calcium and boron in the older tissues of the plant than in the tissue where meristematic activity was highest.

10. The calcium content of the tissues was determined to a large degree by the calcium concentration of the substrate and was largely independent of the nutrient level of boron.

11. Differences in the soluble calcium contents of the top leaves of calcium deficient plants were related to the soluble boron content of the tissues, which in turn were determined by the boron concentration of the substrate.

12. The quantitative relationship between calcium and boron within the plant plays an important role in the metabolic activities of the plant. Boron deficient plants had a high Ca/B ratio, while boron toxic plants had a very low ratio. Plants which apparently were healthy in all respects had intermediate ratios.

13. At a given boron level, increments in the calcium concentration in the substrate caused an increase in the Ca/B ratio of the tissues.

14. At a given calcium level, increments in the boron concentration in the growth media caused a decrease in the Ca/B ratio of the tissues.

ACKNOWLEDGMENT

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